A MANUAL OF

PRACTICAL LABORATORY DIAGNOSIS

BY

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WITH 11 FIGURES AND
8 PLATES—4 IN COLORS

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PREFACE

The author realizes that it may seem superfluous to add another to the very many excellent books of laboratory diagnosis now in use. His purpose is this: It has seemed to him for a long time that all the worth-while laboratory tests that a practical medical man needs, could be put together in a small and compact volume. This should be gotten up in such a way that medical students, and house-officers doing their hospital ward and dispensary work can carry it in their pockets for ready reference, without going to the trouble of hunting through all the various alternative methods of performing a test given in most of the larger books. Much material usually incorporated in laboratory manuals has been left out of this one, and purposely, for many methods and tests which are of scientific value and of theoretical interest, are not *practical* for the average medical man to *use*. It has been the author's aim to include the information that an every day practitioner wants, but to weed out carefully all tests and methods which are not of ordinary clinical application, or which require for their performance complicated apparatus and trained technicians. Thus, such things as tissue staining and fixing, the Wassermann reaction, the gold chloride test, etc. — have been purposely omitted, as these are not ordinarily performed by medical students, house-officers, and general practitioners, for whom this book is intended.

It is a pleasure to thank Dr. John Lovett Morse, Dr. William H. Smith, and Dr. Francis W. Peabody, for their helpful suggestions and criticisms.

For the plates illustrating the sputum we are indebted to Dr. W. H. Smith, and to Mr. L. H. Brown, who made the photographs.

Most of the plates in the chapter on urine were taken from Austin's "Clinical Chemistry."

Boston, *May* 7, 1916.

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CHAPTER I

THE URINE

Collection of Twenty-four Hour Specimen. Empty the bladder at 7 A.M. and throw the urine away. Save all the urine passed up to 7 A.M. the next day, passing it exactly at 7, and adding it to what has already been saved.

Amount. The normal amount of urine is about 1000 to 1500 c.c. in the twenty-four hours. This, of course, is subject to many variations, depending upon the fluid intake, the amount of water lost with the stools, perspiration, etc.

Appearance. Ordinarily a normal urine is clear, but a cloudy urine is not necessarily pathological. Turbidity may be due to:

1. Urates or phosphates (usually normal). Urates are particularly likely to be precipitated in a concentrated urine in a cold room. If due to phosphates, the turbidity clears up on the addition of a little acetic acid; if due to urates, it clears on heating.

2. Pus, or a combination of pus, blood, casts and epithelial cells.

3. Fat (Lipuria). A rare condition. The addition of ether dissolves the fat and renders the urine clear.

Color. The color depends largely upon the amount or urine passed, and upon the percentage of total solids. The normal color may vary from a light

yellow to a rather deep orange. The main abnormal colors are:

1. Pink, due to the precipitation of amorphous urates in an excessively acid urine.

2. Greenish brown, due to bile.

3. "Smoky" or mahogany colored, due to blood cells and casts or to blood pigment and bloody detritus.

4. Brown or black. Urine which when passed is light colored, but which turns dark brown or black on standing (oxidation) usually contains either melanin (in subjects with melanotic sarcoma) or alkapton (a metabolic product).

Specific Gravity. The normal specific gravity varies within rather wide limits — 1015 to 1025. The gravity depends, as does the amount, on several factors, such as fluid intake, loss through sweat, etc. In general a high gravity goes with a small amount, and vice versa. The principal diseases in which a low specific gravity is found are diabetes insipidus (1001 to 1005) and chronic interstitial nephritis (1008 to 1015). The urine in diabetes mellitus is of high gravity (1030 to 1050), due to the presence of sugar.

Reaction. The normal urine is usually acid, the acid reaction being due to acid phosphates in solution. If a urine is alkaline when passed, the alkalinity is most likely to be due to ammonia formation within the bladder, from bacterial activity (cystitis) or to the presence of large amounts of carbonates of the alkalis, which are derived from the oxidation of the salts of the organic acids of vegetable foods. Nearly all urine becomes alkaline on standing, due to bac-

terial decomposition with ammonia formation from the urea in the urine.

Quantitation of the Acidity. In certain cases where there is increased frequency or burning micturition, it is of value to know whether the acidity of the urine is abnormally high.

Folin's Method. To 25 c.c. of urine add a drop or two of a 1 per cent alcoholic solution of phenolphthalein, and 15 or 20 grams of finely powdered potassium oxalate. Shake thoroughly, and titrate with a decinormal sodium hydroxide solution. The acidity is expressed in terms of grams of hydrochloric acid. Each cubic centimeter of decinormal sodic hydrate used is equivalent to .00365 gram of hydrochloric acid. The acidity of the normal twenty-four hour amount of urine corresponds to between 1.15 and 2.3 grams of hydrochloric acid.

ALBUMIN

The ordinary protein occurring in urine is serum albumin.

Nitric Acid Test. To about 15 c.c. of filtered urine in a small urine glass add about 5 c.c. of concentrated nitric acid, letting it run gently down the sides of the glass. A white, flocculent ring at the zone of contact indicates albumin. This test reacts to all urinary protein except peptone. If there is an excess of uric acid in the urine, a light ring similar to the albumin ring is formed, but it is one or two centimeters above the zone of contact, and disappears on gently warming or diluting the urine. Bile pigment produces a greenish ring at the zone of contact. Indoxyl produces a violet ring. A rough idea of the

amount of albumin present may be formed by the thickness of the ring, and may be reported as follows:

1. Slightest possible trace (S. P. T.). — The slightest haze which can be detected, against a dark background.

2. Very slight trace (V. S. T.). — Slightly more.

3. Slight trace (S. T.). — A fairly thick ring, but one which can be hardly seen from above, when looking down through the glass.

4. Trace (T). — Shows a thick, heavy ring, which does not transmit light when looked down upon from above. Not flocculent and not opaque. About .10 per cent albumin.

5. Large trace (L. T.). — Anything more than this.

Heat Test. Fill a test tube one-third full of urine and gently boil the top part of it. If a white precipitate appears in the heated area it may be due to albumin or to phosphates. Add a drop or two of acetic acid. If the precipitate is due to phosphates it disappears, if it is due to albumin it remains.

If a urine contains protein, it is nearly always serum albumin, but such substances as serum globulin, nucleo albumin, mucin and albumose may be present and give the nitric acid or the heat test. For all practical purposes the only one worth considering is albumose. This may be present in the urine of patients with multiple myelomata of the bone marrow. It reacts to the nitric acid and heat tests, but may be distinguished from other proteins by the fact that on heating gently a flocculent precipitate falls at 60° C., which dissolves when the urine is boiled, and reappears when it is cooled.

The quantity of albumin present may be approximately estimated by the method of Esbach as follows: Fill the Esbach tube with filtered urine to the mark U. Then add Esbach's reagent (10 gm. of picric acid and 20 gm. of citric acid in a liter of water) to the mark R, and invert the tube several times. Let it stand over night, and read off from the graduated tube the height of the precipitate. This reading must be divided by 10 to obtain the percentage of albumin.

SUGAR

The only important sugar found in the urine is dextrose (grape sugar). Very small traces of it occur normally, but these small amounts are not recognizable by any of the tests ordinarily in use. Many tests are used for dextrose, but only those which have been found most serviceable by the writer in practice will be given.

In any reducing test for sugar, be sure that no chloroform or formaldehyde has been added to the urine as a preservative.

1. **Fehling's Test.** To 5 c.c. of hot Fehling's solution in a test tube add, a few drops at a time, 5 c.c. of urine, boiling after each addition. If sugar is present, a yellow or a red precipitate of copper oxide is formed.

2. **Benedict's Test.** To 5 c.c. of Benedict's reagent add 8 drops of the urine to be examined. The fluid is boiled from 1 to 2 minutes, and then allowed to cool of itself. If dextrose is present there results a red, yellow or green precipitate, depending upon the amount of sugar present. If no sugar is present the solution may remain perfectly clear or

become slightly turbid, due to precipitated urates. This is a more delicate test than Fehling's.

Fehling's solution consists of two reagents, which are kept in separate bottles and are mixed in equal volumes when ready for use.

(*a*) Copper solution: 34.65 gm. of pure copper sulphate in 500 c.c. water.

(*b*) Alkaline solution: 173 gm. crystallized potassium and sodium tartrate (Rochelle Salt) and 125 gm. of potassium hydroxide dissolved in water and made up to 500 c.c.

Benedict's solution has the following composition:

Copper sulphate....................	17.3 gm.
Sodium citrate.....................	173.0 gm.
Sodium carbonate..................	100 gm.
Distilled water to..................	1000 c.c.

3. **Fermentation Test.** If Fehling's test is positive and there is any doubt as to whether sugar is present or not, the fermentation test is useful as a confirmatory test. About 100 c.c. of urine is put into a small flask and a quarter of a yeast cake is crumbled up into it, and the whole left in a warm place for from 24 to 48 hours. The reduction test is again tried and if negative this time, proves that a fermentable sugar, presumably dextrose, was present in the original sample of urine.

4. **Ozazone Test.** A special property of sugars is the formation of ozazones when treated with phenylhydrazine. Each sugar has its own ozazone, of definite melting point and crystalline configuration.

Add 1 c.c. of phenylhydrazine acetate solution (1 part glacial acetic acid, 1 part water, 2 parts phenylhydrazine, by volume) to 5 c.c. of urine in a test tube, and heat on water bath for half an hour. Allow the liquid to cool slowly, and examine the crystals microscopically (see plate, p. 31).

QUANTITATIVE TESTS FOR DEXTROSE

1. **Benedict's.** Measure with a pipette 25 c.c. of Benedict's solution into a porcelain dish, add 9 or 10 grams (approximately) of solid sodic carbonate, heat to boiling, and while boiling, run in the urine from a burette until a white precipitate forms. Then add the urine more slowly, drop by drop, until the last trace of blue disappears. The urine should be diluted so that not less than 10 c.c. will be required to give the amount of sugar which the 25 c.c. of reagent is capable of oxidizing. A dilution of 1 to 10 usually answers.

CALCULATION. Five divided by the number of cubic centimeters of undiluted urine run in, equals the per cent of sugar.

Benedict's quantitative solution is prepared as follows: Dissolve 9.0 gm. of copper sulphate in 100 c.c. distilled water. (The copper sulphate must be weighed very accurately.) Dissolve 50 gm. anhydrous sodic carbonate, 100 gm. sodic citrate, and 65 gm. of potassium sulphocyanate in 250 c.c. of distilled water.

Pour the copper solution slowly into the alkaline citrate solution. Then pour the mixed solutions into the flask without loss, and make up to 500 c.c. 25 c.c. of this solution is reduced by 50 mgm. of dextrose, or 67 mgm. of lactose.

2. **Fermentation Test.** Take the specific gravity of the 24° urine, put 100 c.c. of it into a flask, and add to it a quarter of a crumbled up yeast cake. Put the flask in a warm place (at about body temperature) and allow it to remain over night. The next morning test a sample of the fermented urine for sugar. If no sugar is present make the urine up to 100 c.c. (to allow for the water that has evaporated) and take the specific gravity again. Multiply the number of points loss in specific gravity by .23. This gives the percentage of sugar in the urine.

OTHER SUGARS IN THE URINE

It is rare to find any sugar but dextrose in the urine, but levulose, maltose, pentose, sucrose, or lactose may occur.

Levulose. Reduces Fehling's, but more feebly than does dextrose.

SELIWANOFF'S REACTION. To 10c.c. of urine add a small amount of resorcin and 2 c.c. dilute hydrochloric acid, mix and heat in a test tube. If levulose is present the liquid turns red and precipitates a dark sediment, which is soluble in alcohol, with the formation of a bright red color.

Maltose. Occurs very rarely in disease of the pancreas, in very small amounts. Of no practical interest.

Pentose. Pentoses reduce Fehling's solution rather slowly. They do not ferment. They may be detected by Bial's orcin test.

BIAL'S TEST. Five cubic centimeters of Bial's reagent (500 c.c. of 30 per cent hydrochloric acid, 1 gm. orcin, and 25 drops of 10 per cent ferric chloride solution) are boiled in a test tube and the urine to be tested is added drop by drop. A green color indicates the presence of a pentose.

Levulose, maltose, and the pentoses occur in urine so rarely as to be of very little practical importance.

Lactose. Lactose may occur in the urine of pregnant or nursing women. It reduces Fehling's solution, but does not ferment with pure yeast, and does not give a dextrosazone. Lactose may be detected by Rubner's test.

RUBNER'S TEST. To 10 c.c. of urine add 3 gm. of lead acetate; filter off the precipitate and heat the

filtrate in a test tube until a yellowish brown color appears, then add a little ammonia and continue heating. If lactose is present, a brick red color appears and a cherry red precipitate settles at the bottom of the test tube, while the liquid above becomes colorless.

Sucrose. May rarely occur in the urine after eating large amounts of saccharine food. It does not reduce Fehling's or Benedict's solutions and is of no clinical importance.

OTHER REDUCING SUBSTANCES IN THE URINE WHICH MAY BE CONFUSED WITH DEXTROSE

Drugs. Certain drugs, such as chloral, naphthol, antipyrin, aspirin, morphine, camphor and menthol, cause an increase in the conjugate glycuronates (glycuronic acid conjugated with aromatic bodies) of the urine. These substances reduce Fehling's solution, but the reduction is usually of a dirty yellow-green color, and rarely the bright yellow or red of a clean-cut sugar reaction. Conjugated glycuronic acid may be distinguished from sugar by the fact that it does not ferment with yeast. Excessive amounts of creatinine or uric acid may cause a partial reduction of copper solution, and may be distinguished from sugar in the same way that glycuronates are.

Alkapton, a product of abnormal metabolism, occurs in the urine rarely and reduces Fehling's solution, but does not ferment. Urine containing alkapton turns dark brown or black on standing, or on the addition of an alkali.

Protein. If a large amount of protein is present in the urine it may cause a reduction of copper solution.

If this is suspected, a few drops of acetic acid should be added to the urine, and it should be boiled a few minutes and filtered to remove the precipitated protein.

ACETONE AND DIACETIC ACID

Acetone. To 5 c.c. of urine add a crystal of sodium nitroprusside, acidify with glacial acetic acid, shake a minute, and then make alkaline with ammonium hydrate. A purple color indicates acetone.

Diacetic Acid. To 5 c.c. of urine add an excess of a 10 per cent solution of ferric chloride. A burgundy red color indicates diacetic acid. After the taking of certain drugs, especially aspirin, a diacetic acid reaction may appear in the urine, which *does not* disappear on heating. The red color if due to diacetic acid, disappears on heating.

QUANTITATIVE TEST FOR AMMONIA

To 25 c.c. of urine add 5 c.c. of a saturated solution of potassium oxalate and 2 to 3 drops of a 1 per cent alcoholic solution of phenolphthalein. Run in from a burette decinormal sodic hydrate, to a faint pink color. Then add 5 c.c. of formalin (40 per cent commercial) and again titrate to the same color. Each cubic centimeter of the decinormal alkali used in this last titration equals 1 c.c. of $n/10$ ammonia, or .0017 gm. of ammonia. Multiply .0017 by the number of cubic centimeters of decinormal alkali used in the titration; this gives the number of grams of ammonia in 25 c.c. of urine. The potassium oxalate and the formalin must both be neutral to phenolphthalein, and the urine must be fresh.

The value of quantitating the ammonia is that it is a rough index to the amount of acidosis in diabetes. The amount of ammonia in the urine varies of course with the amount of protein ingested, but in general an ammonia figure of 1 to 2 grams per 24 hours may be regarded as normal, anything over this indicates an acidosis, anything over 4.0 gm. a severe acidosis.

BILE

The most satisfactory test for bile for general use is the iodine test. To 15 c.c. of urine in a test tube add 5 c.c. of a 1 to 10 dilution of the official tincture of iodine, letting it run gently down the side of the tube. A green ring at the zone of contact of the two liquids indicates bile.

Gmelin's Test. Filtered urine is allowed to slowly trickle down the side of a test tube containing a few cubic centimeters of concentrated nitric acid. If bile is present several colors (green, blue, violet, yellow) are formed at the line of junction of the two fluids.

Hammarsten's Test. A mixture of 19 parts of 25 per cent hydrochloric acid and 1 part of 25 per cent nitric acid is allowed to stand at room temperature for from several hours to a day, until it has turned slightly yellowish. One part of this acid mixture is added to 5 parts of 95 per cent alcohol. A few drops of urine are added to a few cubic centimeters of this acidulated alcohol. If the urine contains bile pigment, a characteristic green color appears almost immediately. This is a delicate test.

A very simple and fairly satisfactory test is to dip a piece of white cotton cloth into the urine. If bile is present the cloth will be stained yellow.

UROBILINOGEN

Half fill a small test tube with urine, and add 3 or 4 drops of Ehrlich's reagent (2 per cent solution of dimethyl-paramido-benzaldehyde in 20 per cent hydrochloric acid) taking care that the reagent remains on top of the urine. A cherry red color, usually appearing within an hour, indicates urobilinogen.

MELANIN

Urine containing melanin is dark when passed, but turns darker on the addition of an oxidizing agent, such as ferric chloride, potassium dichromate or sulphuric acid. An excess of the oxidizing agent decolorizes the urine, with the formation of a yellow precipitate.

Von Jaksch-Pollak Reaction for Melanin. Add a few drops of ferric chloride solution to a third of a test tube of urine, and note the formation of a gray color. Upon the further addition of the chloride a dark precipitate forms consisting of phosphates and adherent melanin. An excess of ferric chloride causes the precipitate to dissolve.

BLOOD PIGMENTS

Guaiac Test (see p. 127).

FORMALDEHYDE

Burnham's Test. To 5 c.c. of urine in a test tube add 5 drops of a .5 per cent solution of phenylhydrazine hydrochloride, a crystal of sodium nitroprusside, and shake. Now add a few drops of a concentrated solution of sodic hydrate. If formaldehyde is present a blue color results, slowly passing through blue to brown,

red, and finally yellow. This test is of importance in examining the urine of patients who are being treated for pyelitis with urotropin. If the test for formalin in the urine is negative it shows that the urotropin is not being broken up in the body, and is consequently doing the patient no good.

INDOXYL

Obermayer's Test. Add 3 c.c. of a 20 per cent solution of lead subacetate to 15 c.c. of urine, and remove the precipitate by filtering. (This is done to get rid of urobilin and any other coloring matters that may be present, and is not necessary unless the urine is very highly colored.) Then add 15 c.c. of Obermayer's reagent (2 per cent solution of ferric chloride in strong hydrochloric acid) and shake the mixture. After shaking, 3 c.c. of chloroform is added, and the mixture is again shaken. Indoxyl, if present, gives a blue color to the chloroform.

METALLIC POISONS

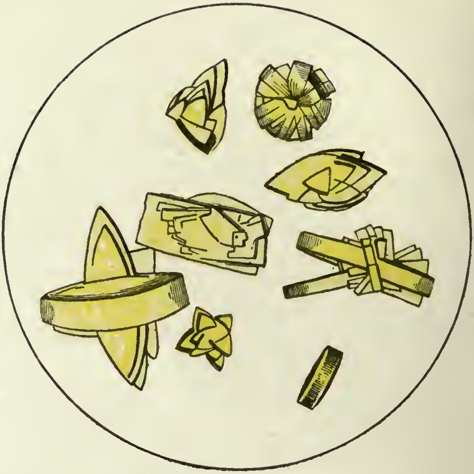
The most common cases of metallic poisoning, that one sees in practice, are those due to lead, mercury or arsenic. Each of these substances appears in the urine, and their quantitative recognition is often a matter of diagnostic or prognostic importance.

Lead. Two liters of urine is evaporated to a tenth of its volume. An equal volume of 20 per cent hydrochloric acid is added, and 3 gm. of potassium chlorate. The mixture is heated on the water bath to 60° C. As soon as the evolution of chlorine has ceased another portion of 3 gm. of potassium chlorate is added, and the operation repeated until the fluid

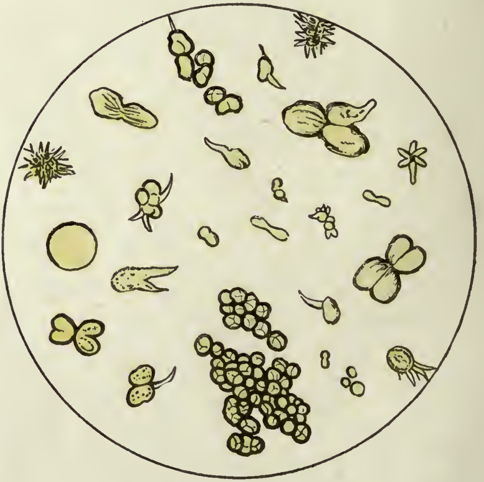
no longer gives off the fumes of chlorine on the further addition of the chlorate. If the liquid becomes too concentrated, more water is added. The fluid is allowed to cool and is filtered after dilution with water. The filtrate is examined for lead with hydrogen sulphide, sulphuric acid, and potassium bichromate, giving precipitates, if lead is present, of black lead sulphide, white lead sulphate, and yellow lead chromate.

Arsenic. May be tested for by saturating the faintly acid urine with hydrogen sulphide gas, allowing to stand from 12 to 24 hours, filtering, washing, treating the precipitate with bromine water, which will dissolve the arsenic sulphide. The solution is placed in a small Erlenmayer flask, to which is added zinc and sulphuric acid, and the stream of hydrogen is conducted into an acid silver nitrate solution ($AgNO_3$ 1 to 2 gm., HNO_3 2 gm., water 10 c.c.). If AsH_3 is generated, one gets a blackish brown precipitate of metallic arsenic.

Mercury (method of Vogel and Lee). "150 c.c. of urine is taken, and in order to break down the organic compound in which the mercury is likely to be present, it is acidulated with 5 c.c. of concentrated hydrochloric acid and evaporated until its bulk has been reduced to 25 or 30 c.c. About 2 c.c. of hydrochloric acid is added to replace the loss by evaporation, and enough potassium chlorate to oxidize thoroughly the organic material present. This usually requires about 2 gm. and when it has been oxidized the fluid becomes pale yellow or colorless. It is then diluted to about 60 c.c. and is boiled vigorously until the chlorine gas previously evolved has been driven off,



Uric Acid Crystals.



Acid Ammonium Urate.

which is shown by the absence of chlorine odor from the steam. The solution usually darkens again on cooling. A piece of copper wire about 4 cm. in length, bent back on itself twice, and cleaned by boiling in a test tube with dilute hydrochloric acid, is dropped into the solution and allowed to remain an hour or more. If considerable amounts of mercury are present it will be found to be coated with a silvery film of metallic mercury; but this is not sufficient to establish the identity of the metal, and if it exists only in traces the changes in the appearance of the urine may be inconclusive. The wire is accordingly removed from the dish with a glass rod, is washed with a little water, and is gently dried by rolling it on a piece of filter paper, pains being taken to avoid unnecessary handling.

"It is then put into the bottom of a glass tube from 3 to 5 mm. in diameter and 15 cm. long, which is sealed at one end and is followed by a cylindrical plug of gold leaf which is pushed into the tube until it is within 2 cm. of the wire. (Such pellets of gold leaf may be obtained from any dentist.) Holding the tube horizontally, the end containing the wire is gradually heated, the part of the tube containing the gold leaf not to be heated. The latter must be examined frequently for any change of color, especially the end of the cylinder toward the wire. If mercury is present it will manifest itself by the appearance of a silver patch of amalgam in this situation.

"If the amount of the metal is very small there will be simply a pale discoloration of the gold, but if the amount is larger, the deposit on the gold will be very easy to recognize. If further confirmation of the

Glucosazone Crystals.

Amorphous Urates.

identity of the mercury is required, the gold-foil may be suspended in a tube containing a few crystals of iodine, which are then very gently warmed. The mercury thus becomes converted into red mercuric iodide, or if the amount is considerable the metal may be distilled by heating the gold foil in the tube and looking with the lens for a deposit of very minute droplets of metallic mercury in the cooler parts of the tube."

SEDIMENTS

1. Unorganized Sediments

Uric Acid. Uric acid occurs in acid urines in a variety of crystalline forms. Some of the more common forms are: "Dumb-bells," "whetstones," hexagonal plates, rhombic prisms or wedges. The crystals are usually yellowish in color. Uric acid crystals are soluble in alkalis, and hence do not occur as a sediment in alkaline urine. They have little clinical significance, unless associated with blood or other evidence of renal or ureteral irritation, when the possibility of calculus formation should be borne in mind.

Urates. Urates may occur singly or as a mixture of ammonium, calcium, magnesium, potassium, and sodium urates. Ammonium urates may occur in acid, neutral or alkaline urine, but all the other urates occur as sediment only in acid urine. Calcium, magnesium, and potassium urates are amorphous, ammonium urate is crystalline, and sodium urate may be either amorphous or crystalline. Ammonium urate ordinarily occurs in the form of the so-called "thorn apple," crystals, while sodium urate, when

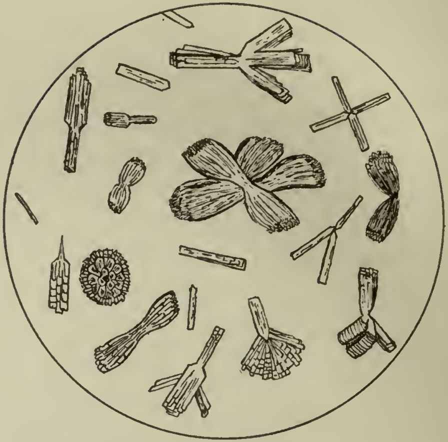


FIG. 1. Calcium Phosphate

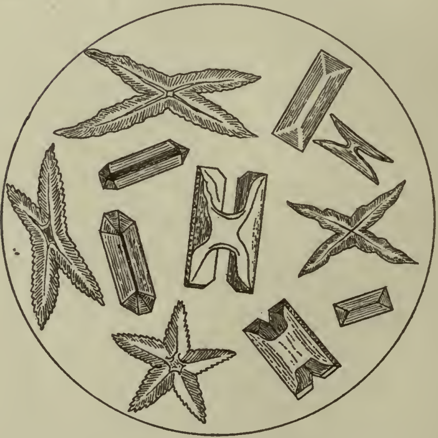


FIG. 2. Ammonio-magnesium Phosphate

crystalline, occurs as fan-shaped clusters or prismatic needles. The urates are all soluble in hydrochloric or acetic acid. *The most common urate sediments seen are the "thorn apple" crystals of ammonium urate, and the so-called "brick dust" pinkish precipitate of amorphous urates.* Urate sediments have little clinical significance, and are especially seen in rather concentrated, highly acid urines.

Phosphates. Phosphates may occur as calcium or magnesium phosphate or as ammonium magnesium phosphate (triple phosphate). *Calcium phosphate* may occur in crystalline or amorphous form. It appears in the sediment of slightly acid, neutral or faintly alkaline urine. The crystalline form is seen in long glistening prismatic needles, which may be arranged singly or in bundles or rosettes. *Amorphous phosphates* (calcium and magnesium) are usually seen in alkaline urines, but may be present if the reaction is slightly acid or neutral. Microscopically they are seen as granular, colorless masses, and are dissolved by acetic acid but not by heat. *Magnesium phosphate* crystals are not common. They are found rarely in alkaline urines in the form of rhomboid plates, which are soluble in acetic acid. *Ammonium magnesium phosphate* (triple phosphate) occurs usually in alkaline urines, and is seen in the form of rhomboid prisms of characteristic appearance, the so-called "coffin lid" form. Occasionally they may closely resemble the large envelope forms of calcium oxalate, but may be distinguished from them by their ready solubility in acetic acid. *The most common phosphates seen are the amorphous phosphates, and the ammonium magnesium phosphate. These two forms*

FIG. 3. Calcium Oxalate Crystals

FIG. 4. Leucin and Tyrosin
1, Leucin and 2, Tyrosin Crystals

commonly occur in urines that have been standing and have become alkaline, and are of little clinical significance. When occurring in freshly passed urine, they indicate ammoniacal fermentation, due possibly to cystitis or to retention of residual urine.

Calcium Oxalate. Calcium oxalate crystals occur in the urine in two forms, the dumb-bell type and the octahedral type. They are found most frequently in acid urines, but may occur in neutral or alkaline urines as well. They are insoluble in acetic acid, but soluble in hydrochloric. Oxalates are derived from various vegetable foods, particularly spinach and rhubarb, and if present in the urine in large numbers are of some clinical importance, indicating the possible formation of a calculus.

Calcium Carbonate. Calcium carbonate crystals are not common in human urine. They occur usually in alkaline urine, but may occur in neutral or very slightly acid urines. The crystals are in the form of granules, spherules or dumb-bells. They may be differentiated from calcium oxalate by the fact that they dissolve in acetic acid with the evolution of carbon dioxide gas. They are of no practical importance.

Calcium Sulphate. Calcium sulphate crystals occur rarely in the urine. They are found usually in very strongly acid urines, are of long needle-like shape, and are insoluble in acetic acid.

Leucin and Tyrosin. Leucin and tyrosin occur rarely in the urine. When they do occur it is usually in the urine of parents with acute yellow atrophy or cirrhosis of the liver, in acute phosphorus poisoning, or in leukemia. They may be in solution, or as a

sediment. Leucin, when crystalline, occurs in spherical masses, which show characteristic radial and concentric striations, and are highly refractive. Tyrosin occurs in the characteristic "sheaf" formation.

TESTS FOR LEUCIN AND TYROSIN. As leucin and tyrosin sometimes occur in solution in the urine, if they are suspected, the urine should be evaporated to a small bulk and the following tests applied.

Leucin. Scherer's Test. To the residue of the evaporated urine add alcohol, which may then be examined for the characteristic crystals. In order to identify the crystals chemically as leucin it is necessary to evaporate with concentrated nitric acid on a platinum crucible cover some of the solid residue obtained after adding alcohol to the evaporated urine. With pure leucin the residue remains colorless, but usually a yellowish residue remains. This is heated with a few drops of sodium hydrate solution, when a yellowish or brownish color appears. If it is heated further, the leucin collects into an oily drop which rolls around on the crucible cover.

Tyrosin. Evaporate the urine to a small bulk, remove the fluid and dissolve the residue in water. Mörner's test is then used. To this aqueous solution add 1 c.c. of a reagent composed of 1 c.c. of formalin, 55 c.c. of concentrated sulphuric acid and 45 c.c. water. When the mixture is heated to boiling a green color appears if tyrosin is present.

Hematoidin. Hematoidin crystals may occur in the urine of persons with various liver diseases. They may be seen as tufts of small needles or as small yellowish red plates.

Fat. Free fat is very rarely seen in the urine. (Lipuria, chyluria.) Before it is decided that the fat droplets seen have actually come from the urinary tract, all sources of contamination must be excluded, such as grease in the vessel in which the urine is obtained, oil on the fingers when manipulating the cover glass, dirt on the slide, etc. Lipuria is so rare that free fat droplets seen in the urine practically always come from one of these contaminations. Fat droplets may occur quite commonly on fatty casts, however. (See below.)

Starch Granules. Starch granules may occur in the urine of babies who have had dusting powder applied to their buttocks. They may be easily recognized by the fact that they turn blue with iodine.

2. Organized Sediments

1. **Epithelial Cells.** As the genito-urinary tract is lined with epithelium and as this is continually desquamating, a few epithelial cells of various sorts are normally found in the urine. If these cells are found in abnormally large numbers their presence indicates irritation or inflammation of the part of the genito-urinary tract from which they come. Epithelial cells may roughly be divided into four classes.

(*a*) *Small round cells*, about the same size as pus cells. They may be differentiated from pus cells by the fact that they are mononuclear. They may come from the renal tubules, or from the urethra or from the pelvis of the kidney. If they adhere together in clumps thay are probably from the renal

pelvis or the urethra; if they are on casts they are from the renal tubules.

(*b*) *Large round cells* two or three times the size of the small ones may come from the neck of the bladder or the membranous or prostatic urethra.

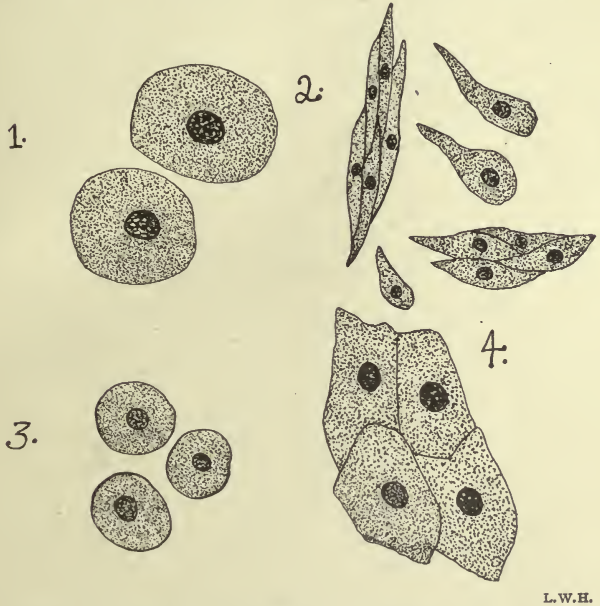


FIG. 5. 1. Large round cells. 2. Caudate cells. 3. Small round cells. 4. Squamous cells.

(*c*) *Large squamous cells* usually come from the the bladder or from the prepuce, or vagina or vulva.

(*d*) *Caudate cells* come from the pelvis of the kidney or from the neck of the bladder or ureter.

2. **Leucocytes** (Pus Cells). A few leucocytes may occur normally in the urine, especially in the urine

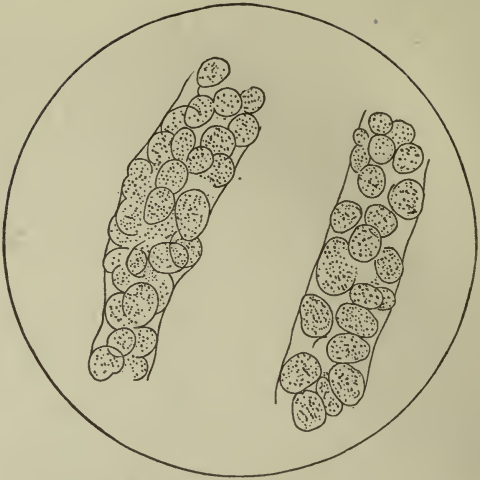


FIG. 6. Epithelial Casts

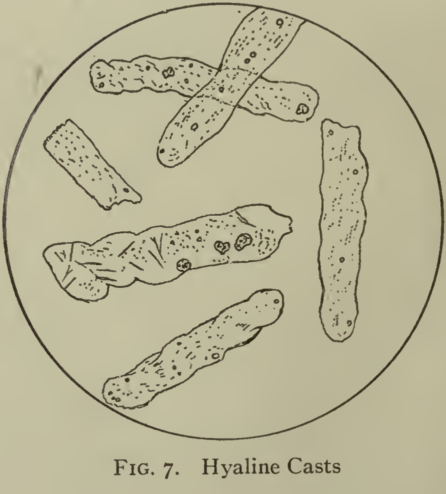


FIG. 7. Hyaline Casts

of females, who are likely to have leukorrhea. For this reason if there is any question of pus in a female's urine, it is desirable to have a catheter specimen to exclude pus from the uterus, cervix, vagina or external genitalia. Be especially careful in young female children and babies, not to make a diagnosis of pyelitis because there are a few pus cells in the urine; these are usually normal. Pus cells may be recognized by their polynuclear nuclei, and these may be made to show more prominently if a drop of acetic acid is run under the cover glass.

3. **Red Blood Cells.** The occurrence of red blood cells in the urine is always abnormal (exclude trauma from the catheter and menstruation). Be careful not to confuse red cells with yeast cells, a great variety of which may appear in stale urine. The yeasts are smaller, are likely to be more irregular or oval in shape and do not show the characteristic refractive outside ring peculiar to red cells, or the flat biscuit shape seen when they are looked at sideways.

4. **Animal Parasites or their Ova.** If there is echinococcus disease of the kidney, small cysts or hooklets from cysts may be passed in the urine. Other parasites which occur very rarely in the urine are the round worms Ascaris and Filaria, and the embryo of Bilharzia (Egypt).

5. **Yeasts and Bacteria.** Normal urine taken by catheter from the bladder is sterile, but on standing many yeasts and bacteria develop, so that if it is desired to determine the presence or absence of a bacteriuria it is necessary to obtain a catheter specimen. The most important bacteria to be looked for in urine are the colon, typhoid, and tubercle bacilli. The

Fig. 8. Fatty Casts

Fig. 9. Cylindroids

colon and typhoid bacilli may be determined by culture, and roughly, by their characteristic morphology in the fresh urine specimen examined under the microscope. To recognize tubercle bacilli a smear of the urinary sediment is made and stained (see p. 159, and be sure to exclude smegma bacilli) or a few cubic centimeters of the urine are injected into a guinea pig. The pig is killed in six weeks, and the presence or absence of tuberculosis determined at autopsy.

6. Casts. Casts have two particular characteristics:

1. One end is rounded, sometimes both ends.
2. Their sides are parallel. (Cabot.)

They may be divided into six classes:

(*a*) *Epithelial casts* are covered with small round cells from the kidney tubules.

(*b*) *Bloody casts* have red blood cells adherent to them.

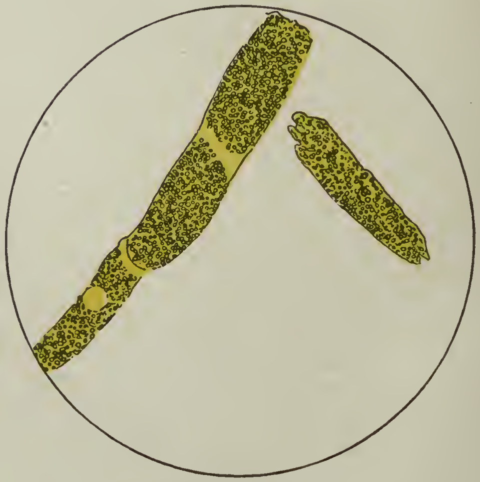
(*c*) *Granular casts* are the most common ones seen; they are covered with coarse yellow granules, probably the degenerated remains of epithelial or blood cells.

(*d*) *Waxy casts* are clear, yellowish or bluish, broader than the other varieties, and are usually seen with broken off square ends, as if they were brittle.

(*e*) *Hyaline casts* are pale and watery looking, and cannot be seen well through the microscope unless the light is shut down.

(*f*) *Fatty casts* have small globules of fat adherent to them, which may be recognized by staining with a drop of Sudan III solution. Their presence means chronic parenchymatous nephritis.

Waxy Casts.



Granular Casts.

Cylindroids consist probably of mucus or mucus-like material. They are longer than casts, more irregular in outline, and have sharp, tapering ends. They are of no particular significance.

SULPHONEPHENOLPHTHALEIN TEST OF KIDNEY FUNCTION. (Rowntree and Geraghty)

One cubic centimeter of the 'phthalein solution * is injected into the muscles of the back, and all the urine passed in the next two hours is saved. A few cubic centimeters of strong sodic hydrate solution is added to make the urine strongly alkaline, the alkaline urine is made up to 1000 c.c. by the addition of tap water, and the red color is compared with a standard, and the result expressed in terms of per cent of this standard.

The large Dubosq colorimeter may be used, or a small one made by Hynson, Westcott Co., or a series of standard test tubes made by oneself.

Directions for Making Standard Tubes. Add a few cubic centimeters of strong sodic hydrate solution to 1 c.c. of 'phthalein solution in a 1000 c.c. graduate, and make up to 1000 c.c. with tap water. This is the 100 per cent solution. The other dilutions may be made up from this as follows: To make an 80 per cent solution take 80 c.c. of the first (100 per cent) solution and add 20 c.c. of water; for a 60 per cent solution, 60 c.c. of the first solution, and 40 c.c. of water, and

* .60 gm. of sulphonephenolphthalein and .84 c.c. of double normal sodium hydrate solution are diluted to 100 c.c. with .75 per cent saline solution, when .15 c.c. more of the double normal sodic hydrate solution is added. The resulting product is filtered, and contains 6 mgm. of sulphonephenolphthalein to the cubic centimeter. This solution may be obtained from Hynson, Westcott Co., Baltimore, put up in very convenient 1 c.c. ampoules.

so on for all the percentages down to 10. The standard solutions are best kept tightly stoppered in large test tubes in a rack in a dark place, as they deteriorate in the light.

The normal function in adults varies between somewhat wide limits: 50 to 75 per cent. In children it is somewhat higher, from 70 to 90 per cent.

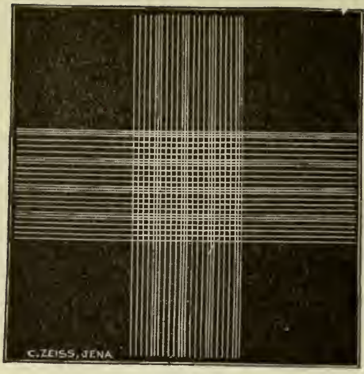
CHAPTER II

THE BLOOD

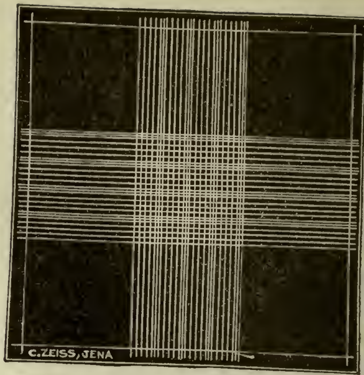
Hemoglobin Estimation (Tallqvist). A drop of blood is secured from the ear, and is allowed to fall upon a piece of dry white absorbent paper. The blood is allowed to stand a minute until it has lost its glistening appearance, the paper is then folded over so there will be a background of white paper against and underneath the blood drop, and the color of the blood is compared with the graded Tallqvist scale. The hemoglobin is read off directly in per cent. Do not hold your finger as a background against the blood on the paper when you are comparing it with the scale; this makes it look too dark. The normal hemoglobin percentage is 80 to 100. This is the simplest of the hemoglobin tests, but is not accurate.

Sahli Method. Draw the blood up to the mark in the small pipette. Then blow it out into the graduated tube, and add $\frac{n}{10}$ hydrochloric acid up to the mark 10. Mix well by a rotary motion. Then add water drop by drop until the color in the graduated tube matches the color in the standard tube. The height of the column of liquid in the graduated tube indicates the percentage of hemoglobin.

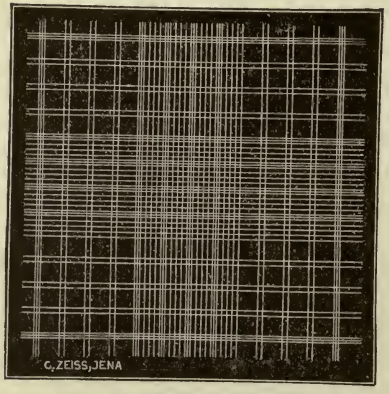
Color Index. The color index is the percentage of hemoglobin divided by the percentage of the normal number of red corpuscles. The normal color index is of course, 1.



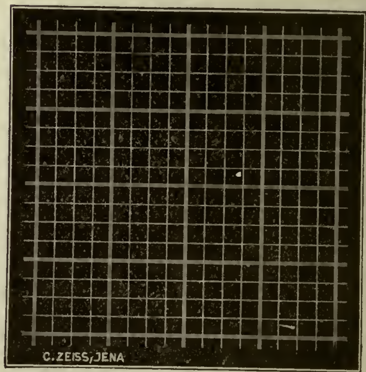
Thoma Ruling.



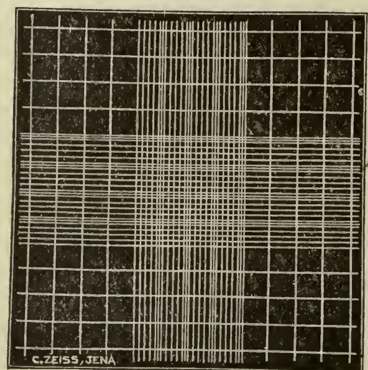
Zappert Ruling.



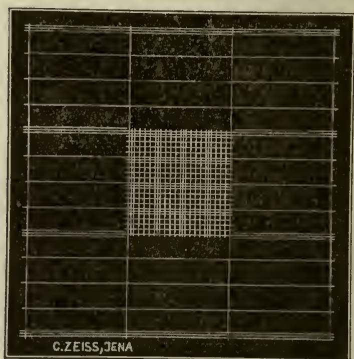
Türk Ruling.



Fuchs and Rosenthal Rulings.



Neubauer Ruling.



Breuer Ruling.

FIG. 10. Diagrams showing various hemacytometer rulings.

Counting the Blood Cells. A number of variously ruled counting apparatuses are in use, but the Thoma-Zeiss is the one most commonly used, and is the one referred to here. The counting chamber is .1 mm. deep. The big ruled square is 1 sq. mm. in area. It contains therefore $\frac{1}{10}$ sq. mm. Each of the smallest squares is $\frac{1}{400}$ sq. mm. in area, and contains $\frac{1}{4000}$ cu. mm.

Count of the Red Cells. The blood is drawn up in the red counting pipette to the mark .5, and then the diluting solution * is added to the mark 101, giving a dilution of 1 to 200. Be sure and shake well, best with a rotary motion, to mix thoroughly the blood and diluting solution. After well mixing, 2 or 3 drops are allowed to run out of the pipette and are discarded, after which a small drop is run on to the counting stage and covered with the cover glass, pressing the glass down with the finger, so that the drop is very evenly distributed. The drop should be large enough to cover the stage when the glass is pressed down. Now count the number of cells (the white cells have been dissolved away by the diluting fluid) in four corner blocks of 25 squares each, add the four counts together and multiply the result by 8000. This gives the number of red cells per cubic millimeter of blood. It is best to count two drops and take the average. The normal red count is about 5,000,000.

Count of the White Cells. Draw up the blood to the mark .5 on the white counting pipette, and follow with diluting fluid (.5 acetic acid) to the mark 11.

* Gower's Solution = sodium sulphate 7 gm., acetic acid 20 gm., water 120 cc.

This gives a dilution of 1 to 20. Put the drop of diluted blood on the slide as in the red count, and count all the cells in the large ruled square. Multiply this count by 200, which gives the number of white cells per 1 cu. mm. of blood. If especial accuracy is desired, it is well to count 2 separate drops, add the two counts together, and multiply by 100.

The normal white count varies from 7000 to 10,000.

COUNTING THE BLOOD PLATELETS

1. Draw the blood up in the red counter just as in doing a red count, to the mark .5.
2. Draw up diluting stain * to the mark 101.
3. Place a drop of blood on the counting stage just the same as for a red count, put on the cover glass, and let stand for a few moments to allow the platelets to settle.
4. Count all the platelets in 100 of the smallest squares, 25 at each corner of the large square.
5. Multiply this count by 8000. This gives the number of platelets in 1 cu. mm. of blood.

The normal platelet count by this method varies from 226,000 to 367,000 (Wright).

The platelets appear as sharply outlined, oval or

* The diluting fluid for counting the platelets consists of 3 parts of a 1 to 1400 aqueous solution of potassium cyanide, and 2 parts of a 1 to 300 aqueous solution of brilliant cresyl blue. These two solutions are kept *in an ice chest* in separate bottles, and are mixed and filtered just before being used. The cresyl blue solution is permanent, but the potassium cyanide should be made up fresh at least every 10 days.

round lilac-colored bodies; the red cells are decolorized and appear as shadows.

A common mistake made is to count the yeasts in the staining fluid instead of the platelets. These are much darker and larger than the platelets are, and if the stain is kept on ice there will be no yeasts. The platelets can be seen better if the specially thin cover glass of Zeiss, with a central excavation, is used.

EXAMINATION OF THE STAINED SPECIMEN

Technique of Making Smear. The best method of making a blood smear is upon small square cover glasses. It is absolutely essential that these be clean, and free from grease. The best method of cleaning them is to soak them for a few moments in concentrated nitric acid, and then to successively wash with water, alcohol, and ether, drying on a soft cloth.

The ear is punctured and the cover glass touched to the resulting drop of blood. Then the second cover glass is dropped over the first, the drop of blood is allowed to spread, and the cover glasses are *quickly* drawn apart, with a sideways, but with no up or down motion. The whole success of a blood smear depends on securing a uniform and very thin film of blood. The important points to observe to attain this are:

1. Do not have too big a drop of blood.

2. Let the drop of blood spread thoroughly, but do not wait long enough before pulling the glasses apart, for coagulation to have started.

3. In pulling the cover glasses apart, use only a

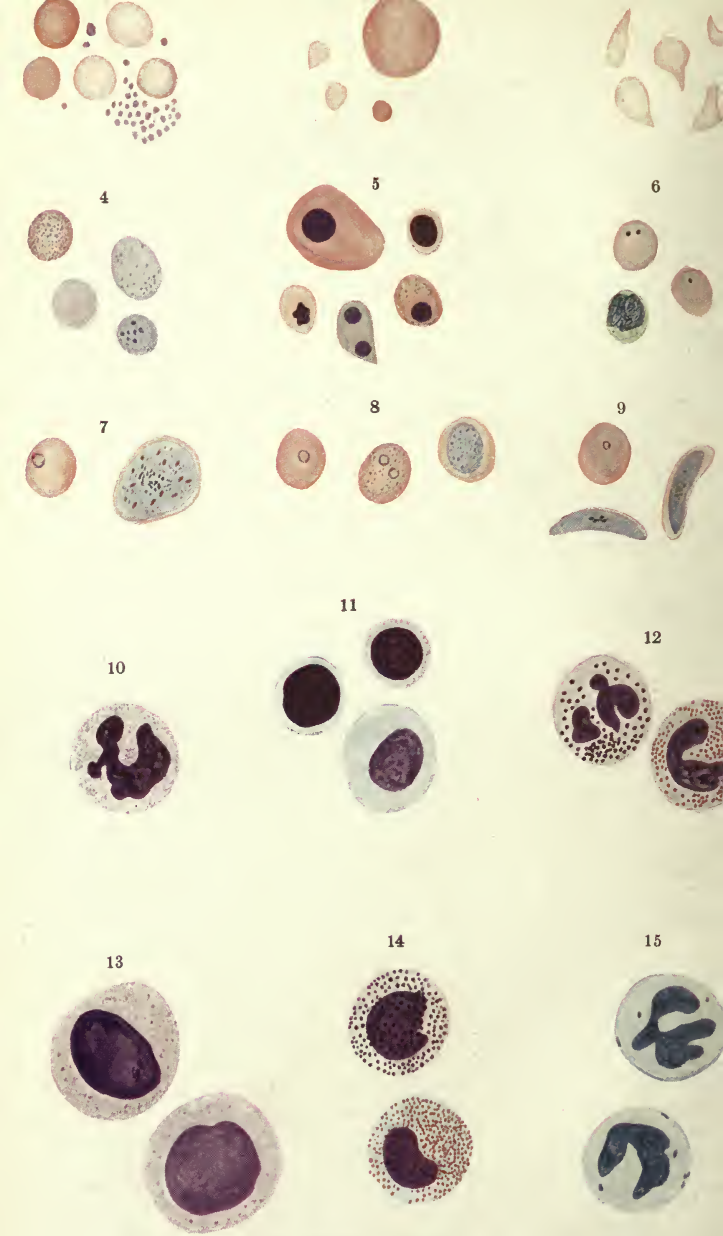
sliding motion; do not *lift* the top cover glass from the bottom one. No fixing, other than drying for a moment, is necessary. The smear is now ready to stain. The most satisfactory stain for general use is Wright's modification of Leishmann's stain. The technique of applying it is as follows (Wright):

1. Cover the film with a known quantity of the staining fluid, by means of a medicine dropper.
2. After one minute add to the staining fluid the same quantity of distilled water, and allow it to remain two or three minutes, according to the density of the stain desired. A longer period of staining may produce a precipitate. Eosinophilic granules are best brought out by a shorter period of staining. The quantity of diluted fluid on the preparation should not be so large that some of it runs off.
3. Wash the preparation in water for thirty seconds, or until the thinner portions of it become yellow or pink in color.
4. Dry and mount in balsam.

THE BLOOD CELLS

The Red Blood Cells. The normal red blood cells appear as nearly round bodies of a fairly uniform size and color, of about 6 to 9 microns in diameter.

The Blood Platelets. These are small bodies, staining purple, about one-half to one-third as large as a red cell, which usually occur in groups. They have been variously considered to be disintegration products of leucocytes, remnants of primitive erythroblastic nuclei, and extrusion processes of large bone marrow cells.



THE BLOOD CELLS.

The Blood Cells

1. Normal red cells.
   Achromic red cells.
   Blood platelets.
2. Macrocyte.
   Microcytes.
3. Poikilocytes.
4. Stippled and basophilic cells.
5. Normoblasts.
   Megaloblast.
6. Cells containing "Howell-Jolly" bodies.
   Reticulated cell.
7. Tertian malaria.
8. Quartan malaria.
9. Estivo-autumnal malaria.
10. Polynuclear neutrophile.
11. Small lymphocytes.
    Large mononuclear cell.
12. Mast cell.
    Eosinophile.
13. Neutrophilic myelocytes.
14. Basophilic myelocyte.
    Eosinophilic myelocyte.
15. Polynuclear leucocytes containing Döhle's inclusion bodies (stained with methylene blue).

The most important types of abnormal red cells are the following:

1. **Microcytes.** Cells smaller than normal.

2. **Macrocytes.** Cells larger than normal.

3. **Poikilocytes.** Cells of distorted shape.

4. **Achromic Cells.** Pale cells with very little coloring matter in the center of the cell, indicating a deficiency in hemoglobin.

5. **Polychromatophilic Cells.** Cells which show a tendency to take the basic portion of the stain, and thus appear of a bluish or a bluish gray color.

6. **Stippled Cells.** Cells containing many fine dark staining granules.

7. **Normoblasts.** Nucleated cells, the embryonic form of red blood cells. These cells may have round nuclei or may have karyorrhectic or multiple dividing nuclei. They may be basophilic and stippled or may take the stain like a normal red cell.

Megaloblasts. Cells of somewhat the same appearance as the preceding, only 4 or 5 times as large.

8. **Skeined Forms.** Cells which when stained with brilliant cresyl blue, show a skeined or reticulated appearance of the protoplasm. They may occur in any anemia, and a few are present in normal blood, but they are especially numerous in chronic family (hemolytic) jaundice. The technique of staining is as follows: To 5 c.c. of a saturated solution of brilliant cresyl blue in .85 per cent salt solution, add 5 c.c. of .85 per cent salt solution and 2 c.c. of a 2 per cent sodium oxalate solution. Filter. A centrifuge tube is now nearly filled with this mixture, and a few drops of blood obtained from the ear are added. The whole is centrifuged for a few moments, washed once

with .85 per cent salt solution, and a smear is made from the sediment of blood in the bottom of the centrifuge tube. The usual tendency is not to get the saturated solution of cresyl blue strong enough; other than this there are no difficulties in the technique.

9. **Howell-Jolly Body Containing Forms.** Cells which contain one or more small black dots, known as "Howell-Jolly" bodies. These bodies are probably portions of the nucleus of the parent normoblast, and occur especially in the blood of pernicious anemia patients after splenectomy has been performed. All of these abnormal forms may occur in severe anemias.

Secondary anemia is characterized by a low color index, therefore considerable achromia. There may be a good deal of tendency to polychromatophilia and stippling, with moderate variations in size and shape (anisocytosis and poikilocytosis). Normoblasts and skeined forms may occur. It is not characteristic of the blood in secondary anemia to have extreme anisocytosis and poikilocytosis, and it is extremely rare to find megaloblasts.

Pernicious anemia is characterized by a high color index, therefore there is usually no achromia. Certain special characteristics of the morphology of the red cells in pernicious anemia are:

1. A tendency to extreme variations in size. Many very small cells will be seen as well as a great many very large ones. The general tendency is to the production of *large* cells, and the occurrence of many very large cells is almost diagnostic of pernicious anemia.

2. A tendency to extreme distortions and varia-

tions in shape. Many elongated and pear-shaped forms are likely to occur.

3. The occurrence of megaloblasts. It is rare to find megaloblasts in a blood of secondary anemia.

Chlorosis is characterized by a very low color index, with consequently a great deal of achromia. There is little tendency to anisocytosis and poikilocytosis, and nucleated forms are rare.

THE WHITE BLOOD CORPUSCLES

The following varieties of white blood corpuscles occur normally.

1. **Polynuclear neutrophiles** constitute about 70 per cent of the white blood cells. They are two or three times the size of a red blood cell, and have irregularly shaped nuclei, with granular, lilac staining, neutrophilic protoplasm.

2. **Lymphocytes** make up about 25 per cent of the white cells. Their usual size is a little larger than that of a red blood cell, although there is considerable variation. Very large forms occur in abnormal bloods, especially in certain cases of acute lymphatic leukemia. The nucleus of a lymphocyte stains dark purple, is usually round or oval, or occasionally indented, and is very clean-cut in its outline. The protoplasm is scanty, showing usually as a narrow ring around the nucleus. It stains light blue, and is usually homogeneous, although sometimes small reddish "azur" granules are seen in it.

3. **Large mononuclear and "transitional" cells** make up from 3 to 5 per cent of the white blood cells. It is best to classify them together. Their exact

status is not definite, but it is probable, although not certain, that they represent one and the same cell, in different stages of development. The large mononuclears are a little larger than the polynuclear neutrophiles, have a single round, oval, or indented nucleus, which takes the stain rather poorly, and a wide zone of basophilic, non-granular protoplasm. These cells may sometimes be confused with lymphocytes. The principal points of differentiation are that the lymphocytes are more intensely basophilic and deeply stained than are the large mononuclears, are a good deal more clean-cut and regular in outline, and do not have such a wide zone of protoplasm around the nucleus. The "transitional" cells are like the large mononuclears, except that the nucleus is indented or horseshoe shaped, and there may be a few neutrophilic granules in the protoplasm. They are called transitional cells, because they were formerly thought to be an intermediate stage in the development of the polynuclear leucocyte. The present view is that they have nothing whatever to do with the polynuclear leucocytes.

4. **Eosinophiles** are usually about the size of a polynuclear neutrophile and constitute about 2 to 4 per cent of the total number of white cells. The nucleus has the same shape as that of the neutrophile, but is likely to appear a little bluer and lighter in color. The protoplasm of these cells is filled with large, round, coarse red granules which take the acid portion of the stain. Sometimes if the diluted stain has been left on too long the granules in the protoplasm of the polynuclear neutrophiles may appear reddish, but they are not nearly so large, red or sharply de-

fined as those in the eosinophiles, and there should be no difficulty in differentiating the two types of cells.

Mast cells (polynuclear basophiles) are cells the size of the eosinophiles, but they show an especial affinity for the basic part of the stain. The nuclei are like those of the polynuclear neutrophiles, except that they usually stain a little more deeply. The protoplasm is filled with large, dark purplish granules. Mast cells form about .5 to 1 per cent of the total number of white corpuscles. They have no particular significance.

ABNORMAL CELLS

Myelocytes. The myelocytes are the primitive forms from which the polynuclear neutrophiles, basophiles and eosinophiles are derived. They do not occur in normal adult blood, but may be seen in certain blood diseases, especially myelogenous leukemia. They vary a good deal in size, sometimes being a little smaller, sometimes being a little larger than the common polynuclear neutrophile. Three types of myelocytes are recognized:

1. **Neutrophilic Myelocytes.** These cells stain rather faintly, are not very sharp in outline, and have a large round or oval or occasionally a kidney-shaped nucleus, and a neutrophilic granular protoplasm. They are differentiated from the other neutrophilic cells by the shape of the nucleus, and from the large mononuclears by their granular neutrophilic protoplasm.

2. **Eosinophilic Myelocytes** are mononuclear cells similar to the preceding, with many eosinophilic granules scattered through the protoplasm.

3. **Basophilic Myelocytes** are similar to those of the eosinophilic form, except that the granules in the protoplasm are basophilic.

THE BLOOD IN INFANCY

The essential fact to remember about the blood in infancy is that it tends more closely to resemble embryonic blood than adult blood does, and that for this reason, certain cells, such as myelocytes and primitive erythrocytes (normoblasts and megaloblasts) may appear in the blood of an infant without having the same significance that they would have in the blood of an adult. In the anemias of infancy, the blood tends to revert to its primitive form, and comparatively slight causes may produce an enormous change in its constitution, with the appearance of many abnormal forms of cells.

QUANTITATIVE DIFFERENCES

(Morse: Case Histories in Pediatrics)

Hemoglobin. The hemoglobin varies between 100 and 125 per cent during the first 3 or 4 days of life; it then drops to the minimum of 60 per cent at three weeks, after which it gradually rises to 70 per cent at six months. It remains at this point during the rest of the first two years, after which it slowly rises, reaching the adult standard at about 6 years.

Red Blood Cells. During the first few days of life the red cells number 6,000,000 to 7,500,000 per cubic millimeter, then the count rapidly falls to the normal infantile limit of about 5,500,000 which it reaches at about two weeks. During the rest of infancy the blood count remains at about this figure,

and gradually diminishes during early childhood, reaching the adult standard at six years.

White Blood Cells. During the first few days of life there is a marked increase, sometimes up to 36,000. The count rapidly drops to 12,000 or 14,000, where it remains during the first six months. The normal limits during the rest of infancy are between 10,000 and 12,000, and the number from this time on is approximately the same as for the adult, 7000 to 10,000.

In young children there is a relatively greater percentage of lymphocytes and a smaller percentage of polynuclear cells. The following table taken from Chapin and Piseks' "Diseases of Children," shows the percentages for different ages.

Year	Polynuclears %	Mononuclears %
1	35	53
2	38	51
3	42	47
4	47	41
5	52	39
6	52	37
7	53	35
8	54	33
9	55	31
10	60	30

MALARIAL PARASITES

In the examination for malarial parasites, the blood is stained with Wright's stain, as in making an ordinary blood smear, and if the parasites are present, they may be easily recognized. Sometimes, however, it takes a long and patient search to find them. By beginners, artefacts or blood platelets lying on red blood cells are likely to be mistaken for parasites, but

if there is any doubt whatever about the body seen being a parasite, it is probably not one, for they stain very prettily, and stand out against the reddish background of the red blood cell in a very clean-cut manner. The protoplasm of the malarial parasite stains light blue and always contains one or more red granules.

Tertian Malaria (Plasmodium Vivax) appears first in the red cell as a small ring-like body, which soon becomes pigmented, and enlarged so greatly that the infected red cell may be swollen to three or four times its normal size, with only a thin ring of protoplasm showing around the body of the parasite.

Quartan Malaria (Plasmodium Malaria) starts as a small ring-shaped body, soon becoming larger and pigmented, as does the tertian. In the quartan parasite the pigment granules are more likely to be arranged around the periphery of the organism, whereas in the tertian they are distributed all through it. The principal point of differentiation between the two forms is that red cells infected with the quartan form do not swell to the large size attained by those containing the tertian parasite, usually remaining about the normal size, or even shrinking a little.

Estivo Autumnal Malaria (Plasmodium Falciparum). The estivo-autumnal form appears in the red cells first as a very small ring-shaped body much like the ring forms of the tertian and quartan, only smaller. The ring is thicker at one side than the other, thus giving it the so-called "signet ring" appearance. The last stage of the estivo-autumnal parasite may occur in the characteristic "crescent" shape, which easily differentiates it from the tertian and quartan forms.

DÖHLÉ'S LEUCOCYTIC INCLUSION BODIES

These bodies are small coccus or bacillus-shaped bodies occurring in the polynuclear leucocytes in a number of diseases, especially in scarlet fever, and were first described by him in 1911. They may be stained with the ordinary Loeffler's methylene blue, but the best stain for them is Granger and Pole's modification of Manson's stain, prepared as follows: methylene blue 1.5 grams; absolute alcohol 10 c.c.; 5 per cent aqueous solution of phenol 100 cc. Methyl alcohol is used as a fixative, it being sufficient to dip the blood smear into the alcohol, wash with water and immediately stain. With this stain the inclusions stain a deep blue, the cell nucleus a deep blue, and the cell protoplasm a pale homogeneous blue.

The inclusion bodies have the following significance:

1. They are present in a majority of cases of scarlet fever up to the tenth day of the disease; in nearly all cases before the fourth day.

2. They are not specific for scarlet fever, being found in a number of the infections, most particularly erysipelas, sepsis, pneumonia and tonsillitis. They are more likely to be found in diseases with which the streptococcus is associated.

3. They have the following diagnostic value: If they are not found in a doubtful case which has a rash and a marked fever, the case is probably not one of scarlet fever.

4. They are probably in the nature of a reaction of injury of the cell nuclei, caused by bacteria toxins, and are small fragments broken off from the nuclei.

WIDAL SERUM REACTION FOR TYPHOID

The ear is punctured and a few drops of blood are allowed to run into a small test tube. This is allowed to stand until the clot and the serum have separated. Now place 9 drops (platinum loop) of an active motile twelve to twenty-four hour old culture of the bacillus typhosus on one end of a glass slide, and add one drop of the serum, mixing well with the platinum loop. Place four drops of water at the other end of the slide, and then add to it one drop of the 1 to 10 dilution which has already been made, making a dilution of 1 to 50. Cover with cover glasses and let stand one hour. Controls should be made by the same method as given above, except omitting the blood serum. If the reaction must be done with dried blood (as in the board of health outfits) add a drop or two of water to the dried blood and proceed as before. Widal's reaction is positive when there is complete agglutination and loss of motility in an hour, in the 1 to 50 dilution. The performance of the test is very easy, but it is sometimes hard to know whether to call a reaction positive or not. Assurance in this can come only after having performed a few.

A and B para typhoid (done the same as typhoid).

The Widal reaction appears usually only after the first week of the disease, and may persist for years in the blood of patients who have had typhoid fever.

BLOOD FRAGILITY TEST

A determination of the resistance of the red blood cells to hypotonic salt solution is sometimes of value in the diagnosis of "chronic family jaundice" (hemo-

lytic), and especially in the differential diagnosis of jaundice due to this disease and that due to obstruction of the bile passages, such as occurs in cases of gallstones, cancer of the pancreas, etc. The red cells in hemolytic jaundice show a lessened resistance to hypotonic salt solution; in obstructive jaundice an increased resistance. The method used by the writer is as follows:

About 6 c.c. of blood is drawn from the arm with a needle and glass syringe. This is transferred to a test tube half full of 0.5 per cent sodium citrate solution in 0.9 per cent sodium chloride. The tube is then inverted two or three times to insure proper mixing. As soon as possible (certainly within three hours) the blood is centrifuged and the cells washed twice with 0.7 per cent sodium chloride. As much of the supernatant fluid as possible is drawn off with a pipet, and the remaining blood cells are used in the test, without further dilution. The hypotonic sodium chloride solutions are made up from a 1 per cent solution of chemically pure sodium chloride and distilled water. The solutions run in strength from 0.70 to 0.175 per cent, and are kept in tightly corked 100 c.c. bottles. Exactly 1 c.c. of each one of these solutions is drawn off in a pipet, and placed in series of small test tubes; 0.05 c.c. of the blood cells is then run into each tube, from a small accurately graded pipet, each tube is inverted twice and allowed to stand two hours at room temperature; at the end of this time the tubes are read. As initial hemolysis take the point at which there is the first tinge of pink in the salt solution; as complete hemolysis the point at which there can no longer be seen any sediment of blood

cells in the bottom of the tube. The average points of hemolysis as obtained by this method are:

Normal blood....	.457	(initial)	3.40	(complete)
Secondary anemia	.475	(initial)	.322	(complete)
Pernicious anemia	.477	(initial)	.322	(complete)
Chronic family (hemolytic) jaundice	.600	(initial)	.400	(complete)
Obstructive jaundice	.400	(initial)	.225	(complete)

HEMOLYSIS TEST

(To Determine the Compatibility of Donor's and Recipient's Blood in Transfusion)

Donor. Secure two samples of blood of 5 c.c. each, from the arm vein. One of these samples is placed in a dry test tube and allowed to clot (to obtain the serum), the other sample is placed in a test tube half full of .5 sodium citrate in 90 per cent sodium chloride (to prevent clotting).

Recipient. The same is done with the recipient's blood. The test is carried out as follows (Lindeman): "The red blood cells of recipient and donor are washed three times with normal saline; variable quantities of recipient's serum are placed in three separate small test tubes. To each of these is added 0.25 c.c. of a 2 per cent suspension in normal saline of washed blood cells of the donor. The same is done with the donor's serum and the recipient's cells. Controls are made of donor's serum and donor's cells — recipient's serum and recipient's cells. Controls are also made with donor's cells in normal salt solution and recip-

ient's cells in normal salt solution. The total volume in each tube is raised with normal saline to 0.5 c.c. The test tubes are incubated in a water bath for a period of two hours, and readings are made. They are then set in an icebox over night and readings are again made the following morning. When the case is urgent the icebox test is eliminated. The icebox test should be eliminated only when absolutely necessary by the extreme condition of the patient where time is the important factor. When the amount of blood taken from the patient for tests is small, only 0.25 c.c. of serum is used and controls of patient's serum are eliminated."

COAGULATION TIME

(Method of Lee and White)

This is a very simple and fairly accurate method. It is performed as follows: One cubic centimeter of blood (approximately) is withdrawn from the arm vein by a small needle and syringe, both of which have previously been washed with normal salt solution, and is put into a small tube of about 8 mm. in diameter, which has also been previously washed in normal salt solution. The time of withdrawal is accurately noted; and the tube is inverted gently every 30 seconds until the blood will no longer run down the sides of the tube, but remains in the bottom of the tube in a solid clot. This is the end point. By this method the coagulation time for normal blood is from 4 to 8 minutes.

OBTAINING BLOOD FOR CULTURES, ETC.

At the present day there are so many serological blood tests and cultures being made, that every practitioner should be able to withdraw the necessary amount of blood from the veins with the least possible discomfort to the patient. A few suggestions may be of service.

Do everything as quickly as you can; it irritates the patient to see you fussing around with your needles and syringes.

Apply a turniquet of small rubber tubing to the upper arm, tightly enough to make the veins of the elbow stand out, but not tightly enough to stop the pulse at the wrist.

Have the patient's arm resting on a table. Use a 5 or 10 c.c. all glass syringe, with a small needle, it is useless to torment the patient with a large one. Wash off the arm at the bend of the elbow *gently* with a little alcohol, with the fingers of the left hand hold the skin tightly on the stretch, and insert the needle with the right, taking care not to run it in too deeply.

Now gently and slowly pull the piston back, and if the needle is in the vein and the syringe is tight, there will be no difficulty in getting as much blood as desired. *Use a sharp needle,* and be sure that it fits the syringe, and that the syringe is tight. If it is not you will be troubled with air bubbles.

In the case of a very fat patient where the veins cannot be seen, the small needle is inserted in the same way, and gently prodded around in all directions until a vein is struck (most of the pain is in the first skin puncture).

In the case of babies the veins of the scalp or the jugulars may be used. A practiced operator can secure enough blood for a Wassermann in a very few seconds, and can save the patient a great deal of discomfort by obtaining the required amount in the shortest possible time.

CHAPTER III

FECES

Color. The most important abnormal colors are:

1. Grayish black — due to the ingestion of iron or bismuth compounds.

2. Tarry black, from digested blood (seen in bleeding cancer or ulcer of the stomach).

3. Green — from calomel.

4. Red — from blood coming from the rectum or low down in the large intestine.

5. Clay colored — from an excess of fat and an absence of bile (obstructive jaundice).

Reaction. Normally neutral or slightly acid or alkaline; an excessively acid stool is due to carbohydrate fermentation, a strongly alkaline one to protein putrefaction.

Consistency and Form. The normal may vary a good deal. The longer a stool stays in the colon or rectum, the harder and drier it becomes, owing to the absorption of water from it through the intestinal wall. In abnormal stools all degrees of consistency may be seen, from the very watery thin ones occurring in cholera, to the round, hard, bullet-like ones seen in certain cases of constipation with an impacted rectum.

MACROSCOPIC EXAMINATION

Spread out a small amount of feces with a little water on a plate.

1. **Abnormal food residue** may consist of cellulose or meat or jelly-like starch remains.

2. **Mucus.** Any but the smallest amount of mucus is abnormal, indicating catarrh or irritation somewhere in the intestinal tract. Mucus from the large intestine is usually present as a thin coating over the outside of the stool — that from the small intestine is well mixed with the fecal matter. Do not confuse soft food residues with mucus.

3. **Blood.** Blood from the rectum usually appears as bright red streaks on the outside of the stool, that from the rest of the lower intestinal tract is intimately mixed with the stool, and gives it a dull brick color — or may appear in streaks (as in amebic dysentery). Blood from the upper intestinal tract or stomach, if present in sufficient amount, gives a dark black color to the stool.

4. **Pus** may appear as yellowish masses or streaks.

5. **Shreds of intestinal mucus membrane** — from ulcerative disease of the intestine.

6. **Gallstones** usually may be recognized by their hardness, and characteristic shape, with facets.

7. **Intestinal sand** consists of small granules of gray or reddish-brown gritty material occurring rarely in the stools. Its origin is not certain. "False intestinal sand" usually consists of the seeds of bananas, or the hard portions of fruits, covered with calcium salts. Neither variety has any particular practical significance.

INTESTINAL PARASITES

(Adapted partly from Kilgore)

The most common intestinal parasites are:

1. **Ascaris lumbricoides** (the common "round worm") varies in length from 15 to 40 cm. It occurs usually in small numbers, and there should be no difficulty in recognizing it, as it is much larger than any of the other ordinary round worms seen in the feces.

2. **Oxyurus vermicularis** (the ordinary pin-worm) is a small worm 3 to 10 mm. long, and usually occurs in the rectum or colon.

3. **Uncinaria duodenalis** (hookworm) is 8 to 18 mm. long. The buccal cavity has three pairs of inward curving hook-like teeth (uncinaria (necator) Americana has a dorsal and a ventral pair of cutting plates instead of hooks.)

4. **Trichuris trichiura** is 4 to 5 cm. long, two-thirds of the length appearing as a whip-like tail.

5. **Tænia saginata** (beef tapeworm). The head is 1.5 to 2 mm. in diameter, and has four suckers, but no hooks. The ripe segments are 16 to 20 mm. by 5 to 7 mm. The uterus has a multitude of fine branchings. The genital openings are irregularly alternate on the margin.

6. **Tænia solium** (pork tapeworm) is very rare in America. The head is .6 to 1 mm. in diameter, and has four suckers on the sides, and on the end a crown of 22 to 32 hooks. The ripe segments are 9 to 10 by 4 to 5 mm. The uterus consists of a large median stem with but 7 to 10 coarse branches, each of which again branches. The genital openings are

at the margin and alternate from side to side with considerable regularity.

7. **Bothriocephalus latus** (fish tapeworm). The head is 1 mm. broad and 2 or 3 mm. long, is flat, almond or spoon shaped, with two deep grooves at its sides. The ripe segments are 10 to 15 by 3 to 4 mm. The genital opening is on the side, not the edge, and the uterus is arranged about it.

8. **Hymenolepis nana** (Tænia nana) is most common in Italy and Egypt, but is occasionally seen in the United States. It is a small worm, only 8 to 25 mm. long, and .5 mm. broad. The head is round and has 4 suckers and 24 to 28 hooklets. They may occur in the stools in extraordinarily large numbers.

CHEMICAL EXAMINATION

Blood. Guaiac test (see gastric contents, p. 127).

Benzidin Test. In a test tube place a small pinch of benzidin, 2 c.c. of hydrogen peroxide, and a few drops of glacial acetic acid. Shake well. Rub up with a glass rod a small portion of feces in another test tube in 5 c.c. of water, and heat to boiling. Pour a few drops of mixture No. 2 into mixture No. 1. If blood is present, a blue-green color results. This is an extremely delicate test, and is of no value unless the patient is known to have been on an absolutely meat free diet for two or three days. The writer never uses this test, much preferring the guaiac test, which is delicate enough to recognize blood in any amount of practical importance.

Bile (hydrobilirubin). Rub up a small portion of feces in a mortar or evaporating dish, with a con-

centrated aqueous solution of corrosive sublimate, and let it stand for 3 hours.

A brick red color indicates the presence of bile.

MICROSCOPIC EXAMINATION

For microscopic examination a small portion of feces is rubbed up with a little water on a glass slide, and examined first with the low and then with the high-power lens.

1. Food Residue

(*a*) **Fat.** Fat may exist in the stool as fatty acids, neutral fat, or soaps; it occurs most commonly as calcium soap, which usually looks yellow under the microscope. Normally there is a certain amount of soap in the stools, while any but the smallest amount of neutral fat is abnormal. Unstained neutral fat appears as small refractile globules, or sometimes with fats of high melting point, as irregular flakes, the fatty acids as flakes or needle-like or plate-like crystals, and the soaps as yellowish masses or occasionally crystals. Fat is best seen when stained with an alcoholic solution of Sudan III. A drop of the stain is added to the rubbed up feces; neutral fat droplets stain bright red, amorphous fatty acids, if present, stain a light red — crystalline fatty acids and soaps do not stain at all. A little more stain and a drop or two of glacial acetic acid is now added, and the slide is gently heated. Neutral fat stays as it was before, soap is broken down to fatty acid and an alkali, and stains in bright red droplets, fatty acid melts and stains in bright red droplets.

Resumé. For neutral fat and amorphous fatty acids, stain with Sudan III alone; for total fat stain with Sudan III + acetic acid and heat.

(*b*) **Starch.** Stain the rubbed up feces with a drop of dilute iodine solution, or with Lugol's solution (IKI). Undigested starch granules stain dark blue, partially digested granules stain a brownish color. The spores of certain fungi may stain blue with iodine, but may be differentiated from starch granules because they lack the characteristic concentric rings, and are smaller and usually oval instead of round. Certain small particles of cellulose may take on a dark color with iodine, but may be differentiated by their irregular shape.

(*c*) **Meat Residue (muscle fibers).** Meat residue occurs in the form of small muscle fibers, which appear yellow. Sometimes these look very much like calcium soaps, but they may be differentiated by the fact that they are striated; soaps are not.

(*d*) **Cellulose** is the fiber structure surrounding vegetable cells. Normally a certain amount of it appears in the feces, and may be easily recognized by its cellular arrangement. With iodine it may stain a dark purplish black, or a dark brown.

Be careful not to confuse round or oval cellular remains with parasitic ova; the ova are always more clean-cut and symmetrical than any cellulose cell.

Unorganized Matter. Various crystals, such as ammonium magnesium phosphate, calcium oxalate, calcium sulphate, cholesterin, etc., occur in the stools, but are of little or no practical importance. After the administration of bismuth salts, irregular crystals

of black bismuth suboxide may be seen. Iron is passed as black amorphous granules.

2. Cellular Elements

(*a*) **Epithelial Cells.** Normally very few epithelial cells appear in the feces. If present in large numbers they indicate an inflammatory condition.

(*b*) **Pus.** A few leucocytes may be found in a normal stool, but are hard to recognize on account of being partly digested. If present in abnormal numbers they may come from inflammatory disease of the intestine itself, or may come from a collection of pus in some other organ perforating into the intestine.

(*c*) **Blood cells** are not commonly seen in the feces unless there is fresh blood present from low down in the intestinal tract. If the blood comes from the upper portion of the tract it is practically always digested.

3. Parasites

(*a*) **Bacteria.** The bacteriology of the stools is an enormous subject and cannot be dealt with in a book of this sort. Two bacteriologic examinations, however, which are of importance and which can be performed so simply that they are of value in ordinary clinical routine are those for the tubercle bacillus and for the "gas" bacillus (Bacillus ærogenes capsulatus).

Tubercle Bacillus. Mix feces and water together in equal parts and centrifuge. Tubercle bacilli, if

present, will come to the top of the tube, and can be stained in the thin scum there. (See p. 159.)

Gas Bacillus (Technique used at the Children's Hospital, Boston).

1. Fill a fermentation tube and a test tube with concentrated nitric acid, let stand 3 minutes, and empty out the nitric acid.

2. Rinse both tubes with hot tap water until neutral to litmus paper.

3. Place a small bit of stool, about a gram of dextrimaltose and about 15 c.c. of hot tap water in the test tube, and boil vigorously for half a minute.

4. Put the contents of the test tube into the fermentation tube, taking care that it is filled up to the top, and that no air bubbles remain in it.

5. Plug the tube with flamed cotton and incubate for 24 hours.

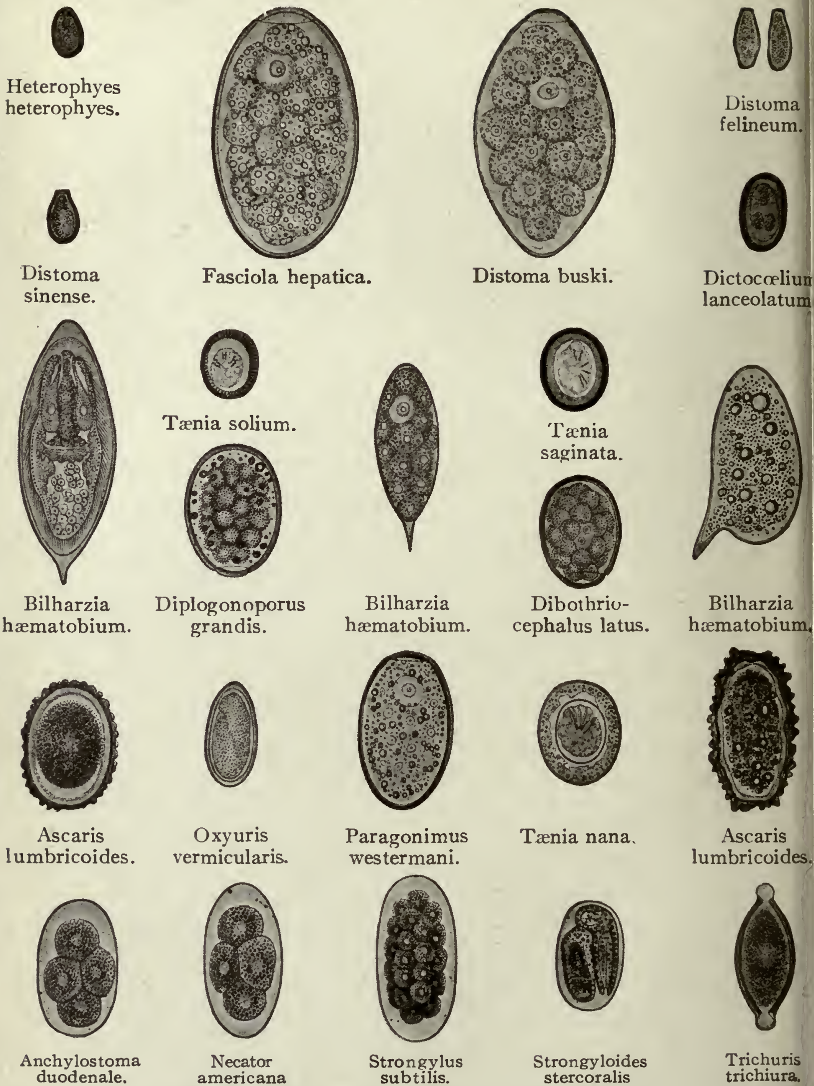
Gas in the top of the tube indicates that the gas bacillus is present, in greater or lesser numbers, depending upon the amount of gas formed.

(*b*) **Protozoa.** In this part of the world the only three protozoans of importance commonly met with in the feces are the *entameba coli*, *entameba histolytica*, and *entameba tetragenus*. Amebæ should be examined for in a fresh stool which has been passed into a vessel containing warm water. If examined for in this way, while still warm, they can often be seen moving about and thrusting out their characteristic pseudopodia, which serve to differentiate them absolutely from any other cells or organisms found in the stool.

TABLE TO DIFFERENTIATE THE THREE IMPORTANT AMEBÆ (CRAIG)

Name.	Size.	Pseudopodia.	Motility.	Protoplasm.	Nucleus.	Pathogenesis.	Staining.
Entameba coli. Schaudinn, 1903.	10–30 microns, usually smaller than entameba histolytica or tetragena.	Small, blunt, and not clearly differentiated from the rest of parasite.	Sluggish.	Ectoplasm not distinct, except when moving. Is grayish in color, and not very refractive. Endoplasm is gray, finely granular, few non-contractile vacuoles. Is not generally phagocytic for red blood cells.	Distinct, having a well-defined nuclear membrane and much chromatin. Large karyosome.	Is not pathogenic, occurring in a large percentage of healthy individuals.	With Wright's stain, ectoplasm light blue; endoplasm dark blue, nucleus red.
Entameba histolytica. Schaudinn, 1903.	10–70 microns, usually from 15 to 40.	Blunt or slender and finger-shaped. Very refractive and clearly differentiated from rest of parasite.	Active.	Ectoplasm is very distinct and refractive, in some instances even when motionless. Glossy appearing. Endoplasm is granular, contains numerous non-contractile vacuoles and red blood cells, when latter are present in feces.	Indistinct. No well-defined nuclear membrane, and but little chromatin. Minute karyosome.	Is the cause of a form of amebic dysentery	With Wright's stain, ectoplasm dark blue; endoplasm light blue, nucleus pale red or pink.
Entameba tetragena. Viereck, 1907.	10–50 microns, about the size of entameba histolytica.	Lobose or finger-shaped. Very refractive and well differentiated from rest of parasite.	Active.	Ectoplasm and endoplasm well differentiated. Ectoplasm hyaline in appearance. Endoplasm granular, containing numerous non-contractile vacuoles and red blood cells, when the latter are present in feces.	Distinct, having definite nuclear membrane formed by chromatin. Large karyosome.	Is the cause of a form of amebic dysentery	Does not stain well with Wright's stain.

FROM CABOT'S PHYSICAL DIAGNOSIS.



DRAWINGS OF EGGS OF INTESTINAL PARASITES.
All are magnified 250. (After Looss.)

(*c*) **Ova of various worms.**

Ascaris lumbricoides. These eggs are usually easily recognized by their rough bile-stained envelope. The protoplasm is unsegmented.

Oxyurus vermicularis. These eggs are small, smooth, oval, and are usually rather light colored. They are more likely to be found on the skin about the anus than in the feces.

Trichiuris trichiura. May be easily recognized by the characteristic light yellow roundish knobs, one on each pole of the egg. These eggs occur often in the stools of people who are perfectly well; the parasites may occasionally cause symptoms, however.

Necator (Uncinaria) americana may usually be recognized by the fact that when seen the protoplasm is likely to be segmented. Often the embryos may be seen curled up within the egg.

Tænia saginata. Usually round or slightly oval — has an outer and an inner shell, with striations between the two.

Tænia solium is very similar to the above; it is very rare in America, however.

Hymenolepis nana. These ova are a little larger than the preceding. They have a double shell, and the space between the inner and outer shells is filled with a granular substance, and is *not* striated.

Bothriocephalus latus. Rather a large egg, larger than the preceding three varieties of tapeworm eggs. They have a lid at one end of the egg, which sometimes may be open, and the contents of the egg looks like a bunch of grapes.

THE STOOLS IN INFANCY

The examination of the stools in infancy is of the utmost importance in determining whether the baby is digesting his food well, and if not, to what particular element of the food the indigestion is due. There is no absolute standard of normality for an infant's stool, it varies so much according to the composition of the food, the relation between the different elements of the food, the nature of the intestinal bacteria, the motility of the intestine, and the absorptive and digestive power of the particular baby in question.

Number. The number of evacuations varies. The normal should not be over four in the twenty-four hours. Breast-fed babies are likely to have more than are bottle-fed babies, owing to the high sugar percentage of breast milk, but, on the other hand, breast-fed babies may sometimes be extremely constipated. Dextrimaltose as a rule is constipating, maltose is laxative — and in general the higher the sugar percentage is in the food, the greater will be the number of stools.

Fermentative and infectious processes in the intestine give rise to an increased number of stools; in fermentative or infectious diarrhea there may be as many as twenty or thirty movements in the twenty-four hours. Nervous influences may cause an increased intestinal peristalsis, and hence an increased number of stools. Sometimes a large amount of insoluble soaps in the stools causes a severe constipation.

Form and Consistency. The infant's stool is usually unformed, and of a mushy consistency. Formed stools, however, may be normal. Constipated stools due to increased soap content are scyb-

alous and crumbly. The longer a stool stays in the rectum the harder it is likely to be. The stools of simple indigestion, or of indigestion with fermentation may vary all the way from a consistency that is slightly thinner than normal, to one that is watery. A common type of stool seen is of the consistency of thin scrambled eggs, with many small white fat curds scattered through it. Fat curds are small, soft, soluble in ether, and are of very common occurrence. Casein curds are large, smooth, white masses from the size of a small bean to that of a large one, are insoluble in ether, and become very hard when put into formalin. They are not of such common occurrence as the fat curds. A foamy, bubbly stool is due to fermentation in the intestine, usually of carbohydrate, but sometimes of protein.

Color. The color varies according to the type of food the baby is being fed on. The typical breast-milk stool is of a golden yellow color. Most stools are usually yellowish, or a dull yellowish brown. Darker brown stools are usually due to an increased protein percentage in the milk. White stools are due to a high insoluble soap content. A stool which is green when passed is almost always abnormal, and is usually due to a sugar fermentation. The green color is due to the presence of biliverdin. Normally bile is present in the stools as bilirubin, which is colorless, but under the influence of intestinal fermentation it is oxidized to the green biliverdin. A stool which turns green on the outside after standing in the air is not abnormal. Black stools are due to the ingestion of bismuth or iron salts, or rarely (in a baby) to digested blood.

Reaction. The determination of the reaction to litmus paper of abnormal stools is of the utmost importance in telling to what food component the indigestion is due. Normal stools may be slightly acid, neutral, or slightly alkaline — a strong acidity or alkalinity usually indicates abnormality. Under *normal* conditions the reaction of the stool depends upon the relation between the fat and the protein, a high fat percentage tending to produce acidity. The stools of normal breast-fed babies are likely to be acid on this account for there is a relatively high fat percentage in breast milk. Under abnormal conditions of fermentation, however, the acid reaction of the stool is due to a high *sugar* percentage in the milk.

In the infant's intestine two processes are always working against each other: decomposition of carbohydrate food, with acid end products, and decomposition of protein food, with alkaline end products. Normally these two processes just about balance each other — when one is greatly in preponderance trouble usually results, and a diarrhea ensues, due to the irritating action upon the intestine of the too strongly acid or alkaline end products. Strongly acid stools due to fermentation of carbohydrate are very common, occurring in the common fermentative diarrhea that one sees so often in summer. Strongly alkaline stools from excessive protein decomposition are not so common.

Odor. The stools of a breast-fed baby usually have a not unpleasant aromatic odor. A high protein percentage in the food causes a rather cheesy and sometimes foul odor. A high sugar percentage, with

fermentation of a part of the sugar, causes an acid odor, sometimes very intense, due to the presence of acetic and butyric acids. The stools in infectious diarrhea may be nearly odorless, or acid, or foul smelling.

Mucus. Any more than the very slightest amount of mucus is abnormal. Mucus may occur around the outside of a constipated stool, owing to its hard character, and consequent irritation of the rectum. Mucus occurs almost always in large amounts in the stools of fermentative diarrhea. This is because of the great irritant action of the volatile fatty acids of sugar fermentation upon the intestine. Mucus also always occurs in infectious diarrhea.

Mucus stools mixed with blood streaks may occur in intussusception.

Blood. Digested blood is rarely seen in the stools of infants. Blood is usually due to infectious diarrhea if it occurs streaked with mucus throughout the stool, or it may occur on the outside of the stool if a fissure of the anus or a bleeding rectal polyp is present.

MICROSCOPIC EXAMINATION

Fat. Technique is the same as for adult stools. (See p. 101.)

Neutral fat rarely occurs, except in very small amounts. Most of the fat residue present in infant's stools occurs as calcium or magnesium soap, and normally not an inconsiderable amount may be present, sometimes as high as a fifth of the weight of the dried stool. It is useless to try to tell anybody, except in very general terms, how much fat should be present

in a normal infant's stool; every observer has to form a standard for himself by repeated examinations.

In "soapy" stools, the "seifen stühle" of the Germans, nearly the whole stool may be made up of soap, and under the microscope the whole field is composed of fat globules when the stool is treated with Sudan III, acetic acid and heat.

Starch. Technique is the same as for adult stools. (See p. 103.)

Starch residue is not commonly found in infants' stools except in cases where too much starch is being fed. The occurrence of more than the smallest amount of starch in the stool is an indication for cutting down the starch in the diet.

Remember that nearly all dusting powders used on babies' buttocks contain starch.

CHAPTER IV

GASTRIC CONTENTS

I

EXAMINATION OF THE FASTING CONTENTS

The fasting contents is obtained through the stomach tube in the morning, when no food *or water* has been taken into the stomach since the previous night.

GROSS EXAMINATION

(*a*) **Amount.** Usually the amount is small — 10 or 15 c.c. Anything over 50 c.c. is distinctly abnormal, indicating stasis or too profuse gastric secretion.

(*b*) **Consistency.** Normally the fasting contents is thin and watery, with a little mucus. An excess of mucus or an increased consistency due to food remains is abnormal.

(*c*) **Color.** The normal color is a light yellowish green, or colorless. A darker green color indicates excessive bile (probably from retching due to the passage of the tube). A chocolate or " coffee ground " color usually indicates digested blood. Fresh blood, when present, is in light red streaks.

(*d*) **Food.** Any macroscopic food residue is abnormal, and indicates stasis, of a lesser or greater degree, depending upon the amount.

(*e*) **Odor.** Normally there is very little odor to the fasting contents; if there is fermentation, the characteristic odors of acetic and butyric acids may be recognized.

MICROSCOPIC EXAMINATION

(*a*) **Food Residue.**

Starch. Stain with dilute iodine solution, or Lugol's solution (IKI). The starch granules stain dark blue.

Fat. Stain with Sudan III. The fat globules appear red.

Normally there may be a very few microscopic particles of food residue; any considerable amount of either fat or starch indicates stasis.

(*b*) **Blood.** Red blood cells may be recognized by their characteristic morphology. Do not confuse them with yeasts.

(*c*) **Pus.** Pus is very rarely found in the gastric contents (unless it has been swallowed with the sputum). Pus cells may be easily recognized by adding a drop of acetic acid to the material to be examined, which makes the characteristic polynuclear nuclei stand out sharply. Pus cells seen in the gastric contents are likely to be partially digested.

(*d*) **Epithelial Cells.** A few epithelial cells may be present in the fasting contents. An excess is abnormal, indicating gastritis.

(*e*) **Tumor Cells.** Occasionally, in cases where there is cancer of the stomach, small pieces of cancer tissue or groups of cancer cells may be brought up by the tube, and sometimes are of great assistance in making a correct diagnosis. If the piece of tissue is

large enough it may be " run through " and sectioned by a competent pathologist, and an exact diagnosis of the sort of tumor present made.

(*f*) **Organisms.**

1. **Sarcinæ** are occasionally seen. They are small cocci, arranged in squares or tetrahedra, and have a very characteristic appearance. They mean stasis, but are rarely found when there is no free hydrochloric acid present.

2. **Yeasts.** Several forms of yeasts of varying sizes and shapes may occur. These may usually be recognized by their tending to show buds.

3. **Boas-Oppler Bacilli.** These are very large, long Gram-positive bacilli, which occur commonly in the gastric contents of patients with cancer of the stomach, especially if there is stasis and lactic acid present. They are rare in non-malignant disease of the stomach.

CHEMICAL EXAMINATION

(*a*) **Free HCl.** A drop of Töpfer's reagent (a .5 per cent alcoholic solution of dimethyl-amido-azo-benzol) is added to a small portion of the fasting contents. If free hydrochloric acid is present a red color results. A still simpler test is with Congo red paper. If a drop of the fasting contents is put upon the red paper, the presence of free hydrochloric acid is indicated by the production of a dark blue color.

(*b*) **Blood.** (Guaiac Test.) To 10 c.c. of gastric contents (or less if 10 c.c. is not available) add a few drops of glacial acetic acid, and 10 c.c. of ether. Shake vigorously. After the ether has separated and has risen to the top, decant. Add this ethereal

extract to a few cubic centimeters of a freshly prepared tincture of gum guaiac. Finally add a few drops of hydrogen peroxide; a blue color indicates the presence of blood. Be especially careful that no blood has come from the teeth, or from abrasions in the mouth, and that no meat is in the fasting contents you are testing. Irritation by the stomach tube, with the consequent production of small hemorrhages, is often given as the source of a positive guaiac test. Practically speaking, the writer believes that it is very rare for the stomach tube to produce enough irritation to cause a positive guaiac test, and most of the cases which he has seen with positive tests, supposedly due to trauma from the tube, have afterwards turned out to have had bleeding ulcer or cancer of the stomach.

II

EXAMINATION OF THE GASTRIC CONTENTS AFTER A TEST MEAL

The test meal ordinarily used in Boston is that of Ewald, and consists of a medium-sized slice of bread, and a glass of water. It is given on an empty stomach, and is withdrawn by the stomach tube at the end of an hour.

Amount. The normal amount is from 50 to 125 c.c. Larger amounts suggest stasis, hypersecretion or hypomotility.

Color. The normal color of the gastric contents after a test meal is the white color of the bread given. If there is digested blood in the stomach, the color will be brown.

CHEMICAL EXAMINATION

(*a*) **Blood.** It is wise to do guaiac tests on both the fasting and the test meal contents.

(*b*) **Free HCl.** The free HCl and the total acidity are determined quantitatively. To exactly 10 c.c. of the unfiltered contents add a drop of Topfer's reagent. If free HCl is present a red color results. Now run in $\frac{n}{10}$ sodic hydrate until the red color changes to yellow. This end point is not a very sharp one, and care must be taken not to over-titrate. Multiply the number of cubic centimeters of $\frac{n}{10}$ sodic hydrate used in this titration by 10. This gives the amount of free HCl present in 100 c.c. of gastric contents in terms of $\frac{n}{10}$ sodic hydrate. The per cent of hydrochloric acid may be obtained if the above quantity is multiplied by .00365. The normal quantity of free HCl is equivalent to between 20 to 60 c.c. $\frac{n}{10}$ sodic hydrate per 100 c.c. gastric contents, or from .07 to .18 per cent.

Total Acidity. To the same specimen of gastric contents in which the free hydrochloric acid has already been neutralized with sodic hydrate, add a drop or two of a 1 per cent alcoholic solution of phenolphthalein. Run in the sodic hydrate, drop by drop, until a faint pink color appears. Multiply the number of cubic centimeters of $\frac{n}{10}$ sodic hydrate used in both titrations by 10; this gives the total acidity of 100 c.c. of the gastric contents in terms of $\frac{n}{10}$ sodic hydrate. The result may be indicated in per cent of hydrochloric acid by multiplying this result by .00365. The normal quantitative values for total acidity are from .15 to .30 per cent or 40 to 80 c.c. $\frac{n}{10}$ sodic hydrate for 100 c.c. gastric contents.

Lactic Acid. Lactic acid is rarely present when free hydrochloric acid is. Its presence indicates the lactic acid fermentation of carbohydrate food, and probably stasis. It may be tested for as follows:

Half fill two test tubes with a very dilute ferric chloride solution. Add a few drops of the gastric contents to one of the test tubes, and compare the color with that of the control. An intensification of the yellow color indicates that lactic acid is present.

Pepsin and Rennin. Pepsin is always present when free hydrochloric acid is. If free hydrochloric acid is absent it may be tested for as follows: To a small portion of the gastric contents add enough hydrochloric acid to make it give the test for free hydrochloric acid, then add a small open tube of coagulated egg albumin to the contents, and leave it in a warm place for 12 hours. If pepsin is present the egg albumin will show signs of digestion.

Rennin also is always present when free hydrochloric acid is. It may be tested for by neutralizing 5 c.c. of gastric contents, adding it to 5 c.c. of milk, and incubating the mixture. If a normal amount is present, the milk will coagulate within 15 minutes.

Mercury. In testing stomach contents or feces the same method is employed as is used for urine (see p. 27), although the quantity of material taken is usually smaller. The material must be well mixed to insure getting a uniform sample of the specimen, especially in stomach contents if egg albumin has been given as an antidote, as the mercury is then in the form of albuminate. The oxidation also takes longer, and larger quantities of potassium chlorate are re-

quired. Before the wire is placed in the solution, the latter should be filtered in order to remove any fatty substances, carbon or other insoluble materials. This test recognizes mercury in a 1 to 100,000 dilution.

CHAPTER V

SPINAL FLUIDS

Pressure. The normal spinal fluid is under very little pressure. Pathological fluids are usually under pressure, the amount depending upon the amount of the fluid.

Appearance. Normal spinal fluid is perfectly clear; pathological may be opalescent or purulent.

Blood may appear in the fluid in cases where there has been a fractured skull or a ruptured blood vessel from other cause; or it may be due to trauma of small blood vessels caused by inserting the needle. Hemorrhage during lumbar puncture is sometimes alarming, but it almost always stops soon if the needle is withdrawn and the patient kept quiet.

Fibrin Clot. In normal fluid, no fibrin clot forms on standing; in pathological fluids a fibrin clot is likely to form, the time of its formation depending upon the intensity of the inflammation and the amount of fibrin present.

Amount. The normal amount obtained by lumbar puncture may vary a good deal, but in general it is not over 20 c.c. In pathological fluids the amount is increased.

In certain cases of meningitis, however, a very small amount, or no fluid at all may be obtained, owing to the formation of adhesions, which prevent the circulation of the fluid.

CHEMICAL TESTS

Pathological spinal fluid usually contains an excess of globulin, the amount depending upon the activity of the inflammation present. This may be tested for as follows:

Globulin

Noguchi's Test. To one part of spinal fluid add four parts of 10 per cent butyric acid in normal salt solution. Boil, and then add as much normal sodic hydrate as there was spinal fluid to begin with, and boil again. Normal fluids may give a faint opalescence, pathological ones are likely to give a flocculent precipitate, indicating an excess of globulin.

Nonne's Test. To about 2 c.c. of spinal fluid in a small test tube, add an equal amount of a saturated solution of magnesium sulphate, and compare this tube with a tube containing spinal fluid alone; a white precipitate indicates an excess of globulin.

Sugar. Normal spinal fluid contains sugar, and gives a prompt reduction of Benedict's reagent. Most pathological fluids do not. In general, the less reduction there is obtained, the more severe is the inflammation. Sugar may be tested for with Benedict's or Fehling's reagent.

MICROSCOPICAL EXAMINATION

1. **Cell Count.** The normal cell count varies. Most normal fluids contain only 3 or 4 cells per cubic millimeter. Authorities differ somewhat as to what is the upper limit of normality in the cell count. A count of over 8 may usually safely be regarded as abnormal.

As the number of cells in a spinal fluid may vary from 1 to 10,000 or more per cubic millimeter, the technique of counting must necessarily be varied somewhat according to the nature of the fluid.

(*a*) **Purulent fluids** are best counted with exactly the same technique used for the white blood cells (see p. 53).

(*b*) **Clear or Slightly Turbid Fluids.** The Zappert modification of the Thoma-Zeiss ruling is the best ruling for the counting slide for clear spinal fluids. In this ruling, besides the large central square, which is the same size as in the ordinary Thoma-Zeiss ruling, there are eight other large squares of equal size around the central square. (See p. 52.)

Technique of Count. Rinse the white blood counting pipette out with glacial acetic acid (to make the cell nuclei stand out), and draw up spinal fluid into it, using no diluting agent. Put a drop of the undiluted spinal fluid on the counting stage the same as for a blood count, and count 5 of the large squares. Do the same with a second drop.

The sum of the two counts equals the number of cells per cubic millimeter.

If a counting chamber with the modified ruling is not available, the ordinary Thoma-Zeiss ruling may be used, as follows: Count all the cells in the large central square in 5 different drops of spinal fluid, and multiply the sum of the counts by 2.

Differential Count. A differential count of the cells may be made at the same time, in the counting chamber, as the acetic acid with which the pipette has been rinsed makes the nuclei stand out very clearly. Normally all the cells are mononuclears.

2. Smear. Smears for the microscopic study of the spinal fluid are made from the centrifuged sediment. It is rather hard to prepare these satisfactorily, but it will be found that if the smear is allowed to dry of itself, without heat, the best preparations will be obtained. The best staining is done with methylene blue. In the stained specimen the following may be seen:

(*a*) **Lymphocytes** — the normal cell of cerebrospinal fluid.

(*b*) **Pus cells** — indicating a purulent inflammation.

(*c*) **Large endothelial cells** — especially likely to be seen in anterior poliomyelitis or cerebrospinal syphilis.

(*d*) **Bacteria.**

1. **Meningococci.** These organisms appear as small, biscuit-shaped diplococci both within and without the leucocytes. They may be present in small or in fairly large numbers.

2. **Pneumococci.** Small or large cocci growing in pairs or in short chains, sometimes showing the characteristic surrounding capsule. When pneumococci are present they usually appear in extraordinarily large numbers, the field under the microscope often resembling a smear from a pure culture of the pneumococcus.

3. **Streptococci and staphylococci** may be easily recognized by their characteristic morphology.

4. **Influenza bacilli** appear as small, thin, rather faintly staining rods, both within and without the leucocytes, in large numbers.

5. **Tubercle bacilli** are present in the spinal fluid in all cases of tubercular meningitis. There are

certain experts who can find them nearly every time, but the average laboratory worker will miss them much more often than he finds them.

The two best methods of looking for them are:

1. Make a smear from the fibrin clot that has formed after the spinal fluid has stood for some time, and stain it for the bacilli. (See p. 161.)

2. Centrifuge the spinal fluid at a high speed for one hour, and make a *thick* smear from the sediment, letting it dry of itself in the air.

CHARACTERISTICS OF THE SPINAL FLUID IN VARIOUS DISEASES

The spinal fluid may vary considerably in different examples of the same disease, or at different periods during the course of the disease. The data given below refer to the *average*.

1. **Tubercular Meningitis.** Almost always slightly opalescent — rarely clear as compared with water. Under slight or considerably increased pressure. May vary in amount from 20 to 125 c.c., and in cell count from 15 or 20 to 1000 cells per cubic millimeter. The cell count is practically never below 10, and the most common count that one sees is somewhere in the neighborhood of 100. Nearly all the cells are mononuclear. The globulin test may be positive or negative, more often positive. A fibrin clot usually forms. Sugar is absent in about 25 per cent of the cases.

2. **Meningococcus Meningitis.** Almost always cloudy, sometimes very thick. The amount is from 5 to 125 c.c., and it may be under slight or considerable pressure. The cell count is high, varying from

200 to 10,000 cells per cubic millimeter. The cells are mostly polynuclear, and the characteristic biscuit-shaped diplococci may be seen both within and without the leucocytes. The globulin test is positive, and a fibrin clot forms if the fluid is not too purulent. Fehling's solution is usually not reduced.

3. **Pneumococcus, streptococcus and staphylococcus** meningitis have much the same sort of fluid that epidemic meningitis does, except that the etiologic organism is likely to be present in greater numbers than is the diplococcus intracellularis in epidemic meningitis.

4. **Influenza Meningitis.** A cloudy fluid, under moderately increased pressure, 25 to 100 c.c. in amount. The cell count varies; in three cases reported by Brown of Toronto, from 1600 to 3600. The cells are nearly all polynuclears, and the influenza bacilli are seen in large numbers both within and without the leucocytes. The globulin is increased, and the sugar decreased or absent.

5. **Poliomyelitis.** Usually under moderately increased pressure, 20 to 100 c.c. in amount. Usually clear. The cell count is moderately increased, from 20 to 50 per cubic millimeter. In the incubation stage, most of the cells are polynuclear, later on in the course of the disease they are mononuclear, up to 95 per cent. Endothelial cells are especially likely to occur. There is usually a slightly increased globulin content, and there may or may not be a fibrin clot. Sugar is usually present.

6. **Encephalitis** is now thought to be the same disease as poliomyelitis, but is confined to the brain. The fluid is the same as that of poliomyelitis.

7. **Serous Meningitis** (Meningismus). Under slightly increased pressure. The amount may vary between 10 and 100 c.c. The fluid is usually clear, has a normal cell count, and reduces Benedict's solution. It may occasionally have a slightly increased globulin content.

8. **Hydrocephalus.** A greatly increased amount, under great pressure. Cell count and globulin normal. Reduces Benedict's. Wassermann may be positive.

9. **Tabes Dorsalis.** A clear fluid under slightly increased pressure. The amount may vary from 10 to 30 c.c. The cell count may vary a good deal, in certain cases being almost normal, and in others considerably increased. The usual count is from 25 to 75. The Wassermann and the globulin tests may be either positive or negative, but are more likely to be negative. Sugar is usually present.

10. **General Paresis.** The amount and macroscopic character of the fluid is about the same as it is in tabes. The cell count is usually a little lower, running from 15 to 50.

The Wassermann and Globulin tests are usually positive. Sugar is present.

11. **Cerebrospinal Syphilis.** There may be a good deal of pressure as well as a considerably increased amount, with a clear or slightly opalescent fluid, depending upon the acuteness and severity of the syphilitic process.

The Wassermann and Globulin tests are positive, the cell count is from 100 to 1500, with a mononuclear formula. Sugar may or may not be present.

CHAPTER VI

PLEURAL AND PERITONEAL FLUIDS

A **transudate** is an accumulation of non-inflammatory fluid in one of the body cavities, due not to infection, but usually to some mechanical disturbance of circulation or to impaired kidney function.

An **exudate** is an inflammatory fluid due to irritation of the serous lining membrane of one of the body cavities by some microörganism.

Transudates. Transudates are usually colorless, or pale straw-colored, of a thin consistency, with a specific gravity of usually under 1018, and an albumin content of below 2 per cent. The sediment shows a few epithelial and endothelial cells, and a very few leucocytes. Transudates very rarely clot.

Exudates. Exudates may vary from a thin consistency like that of a transudate, to a thick, creamy consistency, due to the presence of pus. The color varies from light to dark straw color. The specific gravity is usually over 1018, and the albumin content over 2 per cent. Transudates usually clot spontaneously.

The sediment contains many more cells than does that of transudates, the cells being either lymphocytes or polynuclear cells according to the nature of the infection. The causative microörganism may sometimes be seen in the stained specimen, often in large numbers.

It is often of importance to distinguish between tubercular and other fluids. As a rule, tubercular fluids are clear, and pale, and may be very close to a transudate in some of their properties. There may be few cells in the sediment, or a good many, and these are nearly all lymphocytes. Any pleural or peritoneal exudate which shows a predominance of lymphocytes is presumably a tubercular fluid. As it is not at all easy to demonstrate the tubercle bacillus in tubercular exudates, it is best to inject a few cubic centimeters into a guinea pig, kill the pig after six weeks, and determine whether or not he has developed tuberculosis.

EXAMINATION OF EXUDATES

Albumin.—May be determined by the Esbach tube, as used for urine (see p. 9). As exudates usually have a much higher albumin percentage than any urine that is ever examined, it is best to dilute 1 to 10.

Staining. The staining is best done immediately after the fluid is withdrawn. If it is desired to prevent clotting a few cubic centimeters of a 2 per cent solution of sodium citrate may be added. The fluid is centrifuged and a smear made from the sediment, drying in the air with no heat, and using methyl alcohol as a fixative if one is desired. The best stain is methylene blue, and the important things to observe in the stained specimen are the differential count of leucocytes and the number and variety of any bacteria which may be present.

Purulent fluids may be stained with Smith's stain (see p. 163).

CHAPTER VII

SPUTUM

Source. The sputum may be composed of material from the mouth, nose, naso-pharynx, trachea and larynx, bronchi or lungs. Also, material from diseased conditions in other organs may find its way into the sputum. Examples of this are amebic liver abscesses or pyogenic sub-diaphragmatic abscesses perforating into the lung, or pus from an empyema perforating into a large bronchus.

MACROSCOPIC EXAMINATION

Amount. The amount varies greatly. Especially large amounts are likely to be seen in perforating empyema, pulmonary gangrene or abscess, bronchiectasis, and certain cases of bronchitis. The amount may sometimes reach a liter or more in the twenty-four hours.

Consistency and Appearance. May be very thin or very thick and tenacious. On the basis of the character of the sputum the following classification is usually made.

1. **Frothy** — seen especially in acute edema of the lungs.

2. **Mucoid** — especially likely to be seen in certain cases of bronchitis and in edema of the lungs due to chronic cardiac conditions.

3. **Muco-purulent** — common in many diseases: pneumonia, bronchitis, tuberculosis, etc.

4. **Purulent** — seen in any inflammatory or destructive process within the respiratory tract, where there is pus present (tuberculosis, bronchiectasis, bronchitis, abscess, perforating empyema, etc.). Purulent sputum may be homogeneous, or the pus may be present in small masses, in which case it is said to be *nummular*.

5. **Bloody.** Blood may be seen in the sputum in nearly any inflammatory or destructive process of the respiratory tract. It may exist as bright blood, or dark blood, clotted or unclotted, or it may be present as changed blood pigment. Blood is especially likely to be found in the sputum in pneumonia, where it is very intimately mixed with the thick tenacious sputum, giving it a " rusty " or " prune juice " color, in hemorrhagic infarction of the lung, where the blood is intimately mixed with the sputum, but does not have the rusty color of the pneumonic sputum, or in any destructive process of the lung in which ulceration has occurred (tuberculosis, new growth, gangrene). Blood is also likely to be present in the frothy sputum of acute pulmonary edema, or it may come from ulcerative processes in the throat or esophagus.

Odor. Most sputum is odorless or has a mildly offensive odor. In abscess or gangrene of the lung it may be extremely foul.

Color. Most sputa are light yellow, from mixed pus and mucus. Purulent sputa are a darker yellow, or a rather light greenish yellow. The sputum may be green in jaundice, or in chloroma of the lung. A rusty red or " prune juice " color is characteristic of

lobar pneumonia. The sputum of amebic liver abscess rupturing into the lung has the characteristic brick red "anchovy sauce" color. Gray or black sputum is usually due to inhalation of carbon. A chocolate color may be due to gangrene of the lung.

Casts of the bronchi may be seen in sputum from fibrinous bronchitis, or in cases of diphtheria where the membrane has extended down into the bronchi.

Lung Tissue. In destructive processes of the lung, small bits of lung tissue may appear in the sputum, as dark threads or small masses.

Dittrich's plugs are small, light-colored particles occurring occasionally in the sputum, usually coming from the terminal portions of the small bronchi, or from the tonsillar crypts. They are composed of fatty acids, epithelium and detritus, and have a very offensive odor when crushed.

"**Lung stones**" usually are small masses of calcified tubercular material, and may occur in considerable numbers in the sputum from certain cases of tuberculosis.

Echinococcus Cysts. Portions of echinococcus cysts are sometimes coughed up in the sputum when there is echinococcus disease of the lung.

Food remains of various sorts may be present in the sputum, and should not be confused with any of the pathological constituents.

MICROSCOPIC EXAMINATION

Bacteria

Tubercle Bacillus. Select a thick portion of the sputum, and make a smear from it on a cover glass, drying gently with heat.

1. Cover with Ziehl's carbol fuchsin, and steam for 1 minute, or stain without heat for 5 minutes.

2. Wash in water.

3. Decolorize 20 seconds in Czaplewski's solution (3 per cent strong HCl in 95 per cent alcohol) or in 20 per cent sulphuric acid.

4. Wash in water.

5. Stain with Loeffler's methylene blue 30 seconds.

6. Wash in water, dry, and mount in balsam. The cells, etc., of the sputum stain a light blue, and the tubercle bacilli a bright red. They show an especial tendency to occur in small bundles.

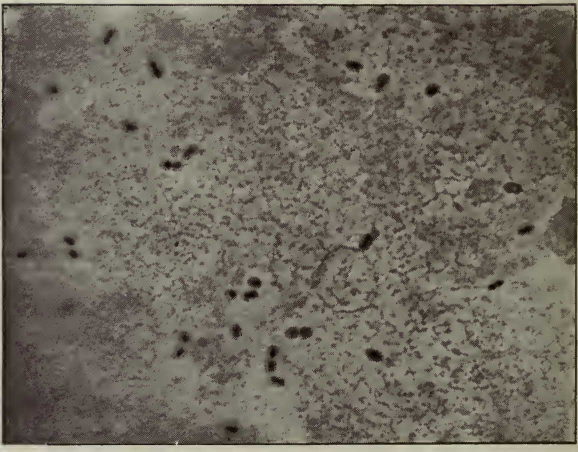
Antiformin Method of Staining. If the bacilli are present in very small numbers they are more likely to be found by the antiformin method, as follows:

To 10 or 20 c.c. of sputum add an equal volume of antiformin (antiformin is a 10 per cent solution of sodium hypochlorite, containing 5 to 10 per cent sodium hydroxide), and mix thoroughly, until the mixture has become homogeneous. All but the acid-fast group of organisms are destroyed. Centrifuge, and stain the sediment for tubercle bacilli.

OTHER ORGANISMS

The sputum contains an extremely mixed flora, and, as many organisms which may be pathogenic are normally present in it, it is unwise to draw too hasty conclusions as to the etiology of a disease from the bacteriology of the sputum. In general, if one organism is present in very large numbers, to the exclusion of the other organisms, it is likely to be the etiologic factor of the disease. Thus, one would pay but little attention to a *few* influenza bacilli in the

Cast of a Bronchus.



Pneumococci.

sputum, but if they were present in large numbers, in nearly pure culture, as they sometimes are, one would expect that they were important in the etiology of the disease.

A very satisfactory stain for all the bacteria in the sputum except the tubercle bacillus, is that of W. H. Smith. In the routine examination of a specimen of sputum, two preparations should always be made; one stained for the tubercle bacillus, and one stained with Smith's stain, as follows:

1. Make a *thin* cover glass smear of the sputum.

2. Stain with aniline oil gentian violet long enough to run through the flame once or twice, steaming gently.

3. Stain with Gram's iodine solution, steaming gently for a moment, as before.

4. Wash with 95 per cent alcohol until no more color comes out.

5. Wash in water.

6. Stain with a 1 per cent aqueous eosin solution for 15 seconds, steaming gently.

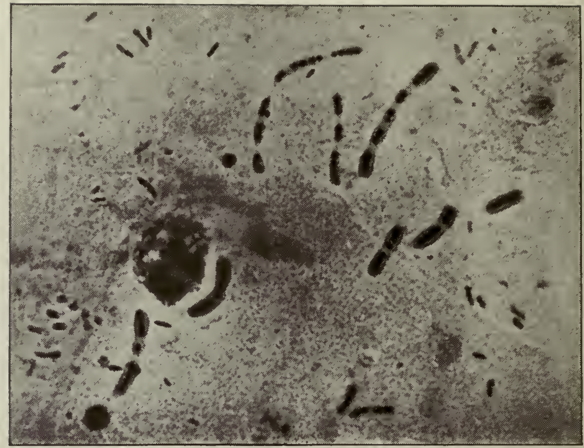
7. Wash in water.

8. Stain with Loeffler's methylene blue for about 30 seconds.

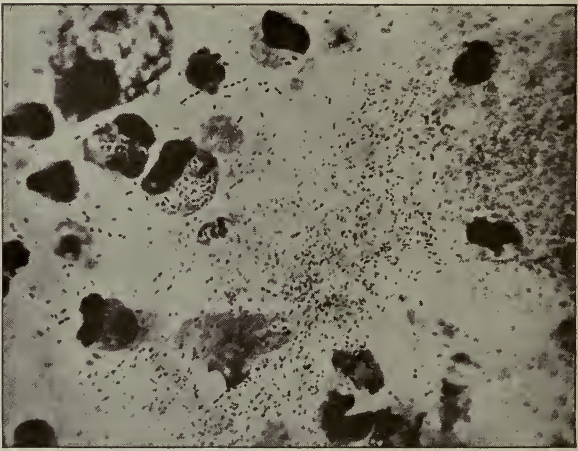
9. Wash in absolute alcohol, followed by xylol, and mount in balsam.

With this stain the Gram-positive organisms appear dark purple, the Gram-negative light blue, and the protoplasm of the cells pink. Capsules stain pink, and eosinophile cells are easily recognized.

Pneumococcus occurs as a small, Gram-positive encapsulated diplococcus, but may grow in chains, which (if the capsule does not show up well) are some-



Streptococcus Mucosus Capsulatus.
(Pseudopneumococcus.)



Influenza Bacilli.

times difficult to distinguish from streptococcus chains. The various groups of pneumococci must be differentiated by their agglutination reactions. Pneumococci are present in nearly every sputum in small numbers, and cannot be regarded as the etiologic organism of the disease unless they are numerous.

Streptococcus is a small Gram-positive organism occurring in chains, long or short. It is seen in many sputa as a secondary infection, but is sometimes the etiologic organism of bronchitis or pneumonia.

Streptococcus mucosus capsulatus (pseudopneumococcus) is a very large, Gram-positive encapsulated diplococcus, appearing usually in large chains. It may be recognized by its large size and distinct capsule. It is a very virulent organism, and if it is the etiologic organism in a case of pneumonia, the prognosis is bad. It is said by some to be present normally in small numbers around the teeth.

Staphylococcus is a small, Gram-positive coccus, appearing in bunches. It occurs to a greater or lesser extent in nearly every sputum, and is sometimes seen in large numbers as a secondary infective agent in the sputum from cases of tuberculosis, lung abscess, bronchiectasis, etc.

Influenza Bacillus. This organism is of considerable importance as an etiologic agent in various respiratory infections, especially in chronic bronchiectasis and sometimes in acute bronchitis or bronchopneumonia. It is a very small, short bacillus, and when it is the cause of a disease it usually appears in very large numbers in the sputum, both within and without the leucocytes. It does not grow on ordinary media, but must be grown on blood agar.

This is prepared by smearing a drop of blood from the finger (taken under sterile precautions) over the surface of the agar in an ordinary culture tube. A loopful of the sputum is planted on this. The influenza colonies appear as very small, clear, " dew drop " like dots, which can best be seen with a hand lens. It is Gram-negative.

Pneumobacillus (Friedländer) occurs as plump rods with rounded ends, varying in size and shape, and often much resembling diplococci. It is Gram-negative, often occurs in pairs, and may have a capsule.

If this bacillus is found in large numbers in the sputum of a case of pneumonia, the patient is very likely to die, as the mortality is always very high in pneumonia of this type.

Micrococcus tetragenus is a small coccus in tetrad form, within a capsule, which is likely to appear in mixed infections. It is Gram-positive. It is not of much practical importance.

Micrococcus catarrhalis is a diplococcus or sometimes a tetracoccus, but does not form chains. It looks very much like a gonococcus, but is larger. It is Gram-negative. At one time this organism was thought to be of a good deal of importance in causing various respiratory infections, especially in children, but is not now considered very important. It may occur normally in the sputum.

MOLDS, YEASTS AND FUNGI

Actinomycosis of the lung is a rare condition. Macroscopically the actinomyces occur as small " sulphur granule " particles in the sputum. When one of these is crushed on a glass slide, and examined

under the microscope, it is seen to consist of numerous rather fine threads, which radiate from a center in a fan-like manner. The extremities of these threads may be club-shaped.

Yeasts may be present in the sputum. They may closely resemble fat droplets, but may be distinguished by their property of budding. They are usually not pathogenic.

Blastomycetes belong to the same general plant group as yeasts. They may cause blastomycosis of the lung, a very rare condition.

They are oval or round in shape, and have a granular protoplasm with a double capsule. This is separated from the protoplasm by a clear zone. Reproduction in the lung tissue is by budding. They are usually found in the sputum in large numbers when the disease is present.

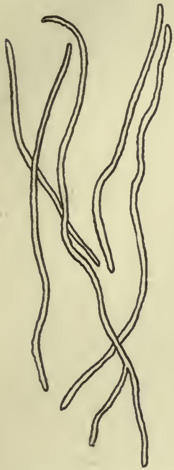
Molds. Are not commonly seen in the sputum, but occur occasionally, as the air is full of them. There are over a hundred varieties. They occur usually as a secondary infection in tubercular or other destructive processes, and are characterized especially by their spores, which are likely to be black, and by their filamentous mycelia.

OTHER BODIES OCCURRING IN THE SPUTUM

Epithelial Cells. As the entire respiratory tract, from mouth and nose to lung alveoli, is lined with epithelium, epithelial cells of various sorts may be found in the sputum. They have little practical importance.

Elastic Tissue. Elastic tissue may be found in the sputum. When found it is an absolute indication

that a destructive process of the lung is going on. It must not be confused with threads of cotton or silk, mycelia of various molds, or elongated fatty acid crystals. True elastic fibers are waxy, extremely

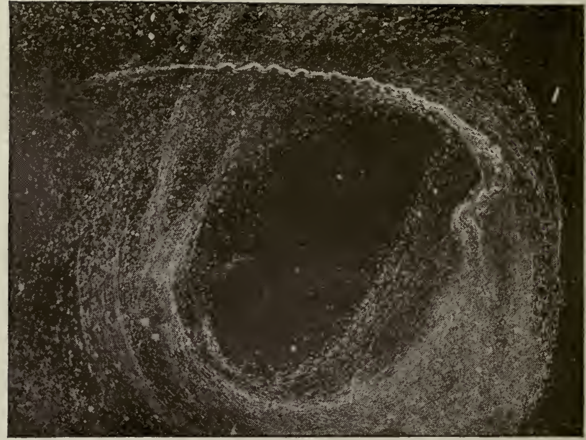


Elastic Fibers.

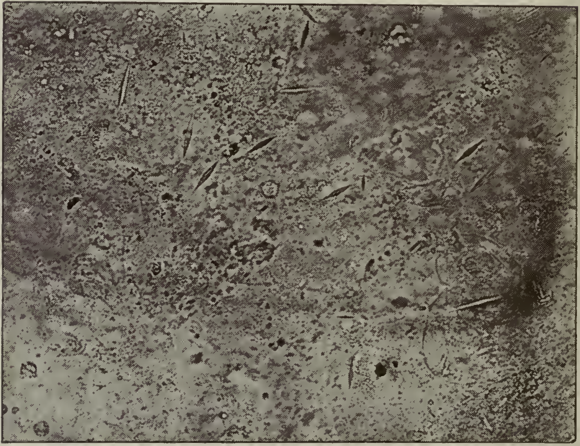
Elastic Fibers in alveolar arrangement.

refractive, usually rather clean-cut in outline, with rounded ends. They may sometimes occur in alveolar arrangement. The best way to look for elastic tissue is to boil the sputum with an equal volume of 10 per cent sodic hydrate until the mixture is homogeneous, dilute with four times its volume of water, centrifuge, and examine the sediment with the low power of the microscope.

Charcot-Leyden crystals, eosinophiles, and **Curschmann's spirals** may be considered together, as they usually occur together in cases of bronchial asthma,



Curschmann's Spiral.



Charcot–Leyden Crystals.

although they may occasionally be seen in the sputum from other diseases.

Charcot-Leyden crystals are seen in nearly every case of bronchial asthma. They are rather long and slender, straight, hexagonal double pyramids, are colorless, and may vary a good deal in size. They are probably derived from disintegrated matter of the eosinophile cells.

Eosinophile cells in the sputum are especially characteristic of bronchial asthma. They are usually mononuclear, not very clean-cut in appearance, and resemble the eosinophilic myelocytes of the blood.

Curschmann's spirals appear as fine collections of threads twisted spirally, usually containing eosinophiles and Charcot-Leyden crystals in the meshes of threads. They are nearly visible to the naked eye, and hence should be looked for with the low power of the microscope. They usually stand out quite sharply against the structureless background of the sputum.

Hematoidin crystals may occur in the sputum either in needles or rhombic form. They are especially likely to be seen in sputum coming from closed cavities containing old pus mixed with blood, such as occur in empyema, lung abscess, amebic liver abscess, etc.

Their color is a bright brownish yellow.

Cholesterin crystals may be seen under the same conditions.

CHAPTER VIII

MISCELLANEOUS

Gram's Stain for Gonococcus.

1. Make a thin smear of the suspected pus on a cover glass.
2. Stain 1 minute with aniline oil gentian violet.
3. Wash in water.
4. Stain 2 minutes with Gram's iodine solution (IKI).
5. Wash in water.
6. Decolorize in 95 per cent alcohol until no more color comes out.
7. Wash in water.
8. Counterstain 1 minute with Bismarck brown.
9. Dry and mount.

The gonococci appear as rather large, biscuit-shaped diplococci, both within and without the pus cells.

Spirochæta Pallida (in primary lesions). Best stained for with Wright's blood stain in the same manner as staining a blood film.

Schick Test. The value of this test is that it shows whether or not a person possesses a natural immunity to diphtheria. One-fiftieth of the minimal lethal dose (guinea pig) of diphtheria toxin, diluted to .1 c.c. with salt solution, is injected *intracutaneously*.

The reaction appears in from 24 to 48 hours after

the injection, and consists of an area of erythema, with a brownish tinge, measuring .5 to 2 cm. It usually reaches its height in 48 to 72 hours, and then fades.

If the reaction does not occur it shows that the patient probably possesses sufficient natural immunity to diphtheria to ward off the infection if exposure should occur.

Von Pirquet (skin) **Tuberculin Test.** With a small awl-like implement the skin of the forearm is scarified in three small places about two centimeters apart. Especial care should be taken not to scarify deeply enough to draw the blood. A drop of old tuberculin is applied to the outside scarifications, the inner one being left as a control, and is allowed to dry a moment, and a light gauze dressing is applied.

The reaction, if positive, usually appears within 24 hours, but should not be called negative until 48 hours have elapsed.

The reaction consists of small, raised, reddened and indurated areas about the points where the tuberculin has been applied.

GRAM-POSITIVE AND GRAM-NEGATIVE ORGANISMS
(Mallory and Wright)

Gram +	*Gram −*
Staphylococcus pyogenes aureus.	Gonococcus.
Staphylococcus pyogenes albus.	Diplococcus intracellularis meningiditis.
Streptococcus pyogenes.	Typhoid bacillus.
Streptococcus capsulatus.	Bacillus coli communis.
Pneumococcus.	Spirillum of Asiatic cholera.
Micrococcus tetragenus.	Bacillus pyocyaneus.
Bacillus diphtheriæ.	Bacillus of influenza.
	Bacillus of glanders.

Gram +

Bacillus tuberculosis.
Bacillus of anthrax.
Bacillus of tetanus.
Bacillus aërogenes capsulatus.
Bacillus of malignant edema.

Gram −

Bacillus proteus.
Bacillus mucosus capsulatus.
Bacillus of dysentery.
Bacillus of bubonic plague.
Bacillus of chancroid.

DISEASES IN WHICH THERE IS A LEUCOCYTOSIS.

Actinomycosis.
Amebiasis (not always).
Appendicitis.
Bronchitis.
Bubonic plague.
Diphtheria.
Endocarditis (acute).
Erysipelas.
Gonorrhea (complicated).
Hydatid disease.
Meningitis (cerebrospinal).
Meningitis (tubercular).
Mumps (lymphocytosis).
Osteomyelitis.
Pancreatitis (acute).
Pneumonia.
Purpura.
Pyelitis.
Rheumatism (acute articular).
Rickets (in severe cases).
Scarlet fever.
Scurvy.
Tonsillitis.
Typhus fever.
Whooping cough (lymphocytosis).

DISEASES IN WHICH THERE IS NO LEUCOCYTOSIS.

Influenza.
Malaria (usually leukopenia).
Measles (usually leukopenia).
Pernicious anemia (leukopenia).
Syphilis.
Tuberculosis.
Typhoid fever (leukopenia).
Variola.